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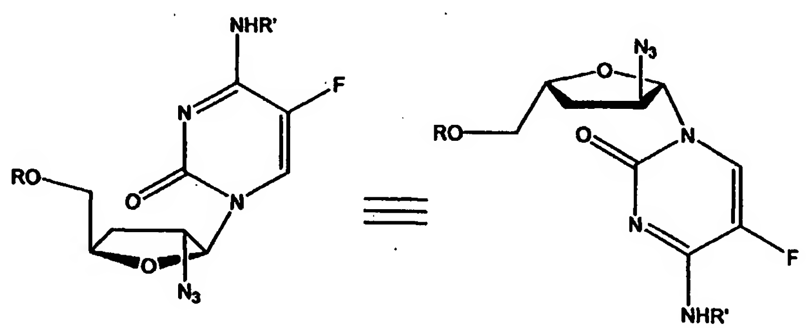

(I)

(57) Abstract

Compounds and pharmaceutical compositions active against hepatitis B virus are provided, as is a method for the treatment of hepatitis B virus infection in humans and other host animals is provided comprising administering an effective amount of a β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine of formula (I) wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

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NUCLEOSIDES WITH ANTI-HEPATITIS B VIRUS ACTIVITY

This invention is in the area of methods for the treatment of hepatitis B virus (also referred to as "HBV") that includes administering an effective amount of one or more of a β-L-2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine to a host in need thereof.

Background of the Invention

HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

Hepatitis B virus has reached epidemic levels worldwide. After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed. Patients typically recover from acute viral hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer. In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is in fact very similar to that of acquired immunodeficiency syndrome, which accounts for why HBV infection is common among patients with AIDS or HIV-associated infections. However, HBV is more contagious than HIV.

Daily treatments with a-interferon, a genetically engineered protein, has shown promise. A human serum-derived vaccine has also been developed to immunize patients against HBV.

Vaccines have been produced through genetic engineering. While the vaccine has been found effective, production of the vaccine is troublesome because the supply of human serum from chronic carriers is limited, and the purification procedure is long and expensive. Further, each batch of vaccine prepared from different serum must be tested in chimpanzees to ensure safety. In addition, the vaccine does not help the patients already infected with the virus.

A number of synthetic nucleosides have been identified which exhibit activity against HBV. The (-)-enantiomer of BCH-189 (2',3'-dideoxy-3'-thiacytidine), known as 3TC, claimed in U. S. Patent 5,539,116 to Liotta, et al., is currently in clinical trials for the treatment of hepatitis B. See also EPA 0 494 119 A1 filed by BioChem Pharma, Inc.

β-2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), claimed in U. S. Patent Nos. 5,814,639 and 5,914,331 to Liotta, et al., exhibits activity against HBV. See Furman, et al., "The Anti-Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (-) and (+) Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-oxathiolane-5-yl]-Cytosine" Antimicrobial Agents and Chemotherapy, December 1992, page 2686-2692; and Cheng, et al., Journal of Biological Chemistry, Volume 267(20), 13938-13942 (1992).

U. S. Patent Nos. 5,565,438, 5,567,688 and 5,587,362 (Chu, et al.) disclose the use of 2'-fluoro-5-methyl-β-L-arabinofuranolyluridine (L-FMAU) for the treatment of hepatitis B and Epstein Barr virus.

Penciclovir (2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6H-purin-6-one; PCV) has established activity against hepatitis B. See U.S. Patent Nos. 5,075,445 and 5,684,153.

Adefovir (9-[2-(phosphonomethoxy)ethyl]adenine, also referred to as PMEA or [[2-(6-amino-9H-purin-9-yl)ethoxy]methylphosphonic acid), also has established activity against hepatitis B. See for example U.S. Patent Nos. 5,641,763 and 5,142,051.

Yale University and The University of Georgia Research Foundation, Inc. disclose the use of L-FDDC (5-fluoro-3'-thia-2',3'-dideoxycytidine) for the treatment of hepatitis B virus in WO 92/18517.

Other drugs explored for the treatment of HBV include adenosine arabinoside, thymosin, acyclovir, phosphonoformate, zidovudine, (+)-cyanidanol, quinacrine, and 2'-fluoroarabinosyl-5-iodouracil.

U.S. Patent Nos. 5,444,063 and 5,684,010 to Emory University disclose the use of enantiomerically pure β-D-1,3-dioxolane purine nucleosides to treat hepatitis B.

WO 96/40164 filed by Emory University, UAB Research Foundation, and the Centre National de la Recherche Scientifique discloses a number of β-L-2',3'-dideoxynucleosides for the treatment of hepatitis B.

WO 95/07287 also filed by Emory University, UAB Research Foundation, and the Centre National de la Recherche Scientifique discloses 2' or 3' deoxy and 2',3'-dideoxy-β-L-pentofuranosyl nucleosides for the treatment of HIV infection.

WO95/13512 filed by Genencor International, Inc., and Lipitek, Inc., discloses the preparation of L-ribofuranosyl nucleosides as antitumor agents and virucides.

WO95/32984 discloses lipid esters of nucleoside monophosphates as immunosuppresive drugs.

DE4224737 discloses cytosine nucleosides and their pharmaceutical uses.

Tsai, et al., in Biochem. Pharmacol. 48(7), pages 1477-81, 1994 disclose the effect of the anti-HIV agent 2'-β-D-F-2',3'-dideoxynucleoside analogs on the cellular content of mitochondrial DNA and lactate production.

Galvez, J. Chem. Inf. Comput. Sci. (1994), 35(5), 1198-203 describes molecular computation of β-D-3'-azido-2',3'-dideoxy-5-fluorocytidine.

Mahmoudian, Pharm. Research 8(1), 43-6 (1991) discloses quantitative structure-activity relationship analyses of HIV agents such as β-D-3'-azido-2',3'-dideoxy-5-fluorocytidine.

U.S. Patent No. 5,703,058 discloses (5-carboximido or 5-fluoro)-(2',3'-unsaturated or 3'-modified) pyrimidine nucleosides for the treatment of HIV or HBV.

Lin, et al., discloses the synthesis and antiviral activity of various 3'-azido analogues of β-D-nucleosides in J. Med. Chem. 31(2), 336-340 (1988).

An essential step in the mode of action of purine and pyrimidine nucleosides against viral diseases, and in particular, HBV and HIV, is their metabolic activation by cellular and viral kinases, to yield the mono-, di-, and triphosphate derivatives. The biologically active species of many nucleosides is the triphosphate form, which inhibits DNA polymerase or reverse transcriptase, or causes chain termination. The nucleoside derivatives that have been developed for the treatment of HBV and HIV to date have been presented for administration to the host in unphosphorylated form, notwithstanding the fact that the nucleoside must be phosphorylated in the cell prior to exhibiting its antiviral effect, because the triphosphate

form has typically either been dephosphorylated prior to reaching the cell or is poorly absorbed by the cell. Nucleotides in general cross cell membranes very inefficiently and are generally not very not very potent in vitro. Attempts at modifying nucleotides to increase the absorption and potency of nucleotides have been described by R. Jones and N. Bischofberger, *Antiviral Research*, **27** (1995) 1-17.

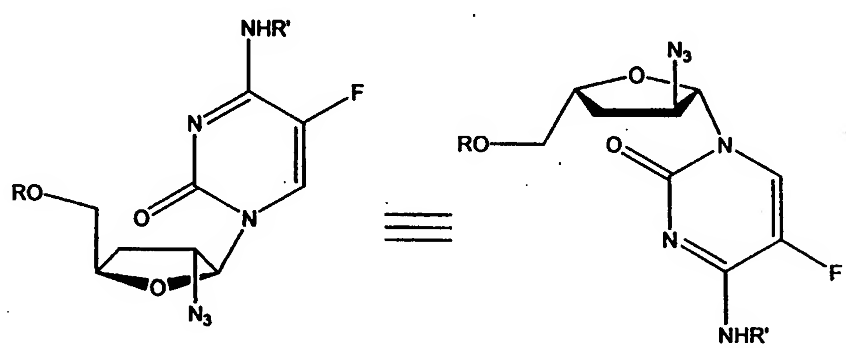
In light of the fact that hepatitis B virus has reached epidemic levels worldwide, and has severe and often tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat humans infected with the virus that have low toxicity to the host.

Therefore, it is an object of the present invention to provide compounds, compositions and methods for the treatment of human patients or other hosts infected with HBV.

Summary of the Invention

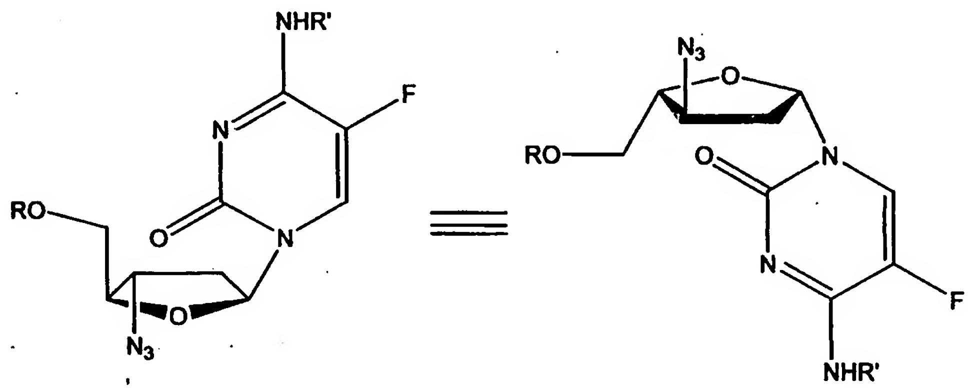
A method for the treatment of HBV infection in humans and other host animals is disclosed that includes administering an effective amount of a β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine nucleoside or a pharmaceutically acceptable salt, ester, or prodrug thereof, including a stabilized phosphate, administered either alone or in combination or alternation with another anti-HBV agent, optionally in a pharmaceutically acceptable carrier. In a preferred embodiment, the 2' or 3'-azido group is in the ribosyl configuration. In a preferred embodiment, the nucleoside is provided as the indicated enantiomer and substantially in the absence of its corresponding β-D-enantiomer.

In one embodiment, the active compound is β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine (L-2'-A-5-FddC) or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

In another embodiment, the active compound is β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine (L-3'-A-5-FddC) or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

The disclosed nucleosides, or their pharmaceutically acceptable prodrugs, esters or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

In one embodiment, the invention includes a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of a prodrug of the specifically disclosed L-(2' or 3')-A-5-FddC nucleosides. A prodrug, as used herein, refers to a pharmaceutically acceptable derivative of the specifically disclosed nucleoside, that is converted into the nucleoside on administration in vivo, or that has activity in itself. Nonlimiting examples are the 5' and N^4-cytosine acylated or alkylated derivatives of the active compound, as well as the 5'-monophosphate, diphosphate, or triphosphate derivatives,

other phosphates, or stablized nucleotide prodrugs, as described in more detail below. For example, the nucleoside is provided as the monophosphate, diphosphate or triphosphate in a formulation that protects the compound from dephosphorylation. Formulations include liposomes, lipospheres, microspheres or nanospheres (of which the latter three can be targeted to infected cells).

In one embodiment of the invention, one or more of the active compounds is administered in alternation or combination with one or more other anti-HBV agents, to provide effective anti-HBV treatment. Examples of anti-HBV agents that can be used in alternation or combination therapy include but are not limited to the cis-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, preferably substantially in the form of the (-)-optical isomer ("FTC", see WO 92/14743); the (-)-enantiomer of cis-2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (3TC); β-D-1,3-dioxolane purine nucleosides as described in U.S. Patent Nos. 5,444,063 and 5,684,010; carbovir, interferon penciclovir and famciclovir.

Any method of alternation can be used that provides treatment to the patient. Nonlimiting examples of alternation patterns include 1-6 weeks of administration of an effective amount of one agent followed by 1-6 weeks of administration of an effective amount of a second anti-HBV agent. The alternation schedule can include periods of no treatment. Combination therapy generally includes the simultaneous administration of an effective ratio of dosages of two or more anti-HBV agents.

In light of the fact that HBV is often found in patients who are also anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV, the active anti-HBV compounds disclosed herein or their derivatives or prodrugs can be administered in the appropriate circumstance in combination or alternation with anti-HIV medications.

The second antiviral agent for the treatment of HIV, in one embodiment, can be a reverse transcriptase inhibitor (a "RTI"), which can be either a synthetic nucleoside (a "NRTI") or a non-nucleoside compound (a "NNRTI"). In an alternative embodiment, in the case of HIV, the second (or third) antiviral agent can be a protease inhibitor. In other embodiments, the second (or third) compound can be a pyrophosphate analog, or a fusion binding inhibitor. A list compiling resistance data collected *in vitro* and *in vivo* for a number of antiviral compounds is found in Schinazi, et al, Mutations in retroviral genes associated with drug resistance, *International Antiviral News,* Volume 1(4), International Medical Press 1996.

Preferred examples of antiviral agents that can be used in combination or alternation with

the compounds disclosed herein for HBV therapy include 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC); the (-)-enantiomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (3TC); carbovir, acyclovir, interferon, L-FMAU, and β-D-dioxolane nucleosides such as β-D-dioxolanyl-guanine (DXG), β-D-dioxolanyl-2,6-diaminopurine (DAPD), and β-D-dioxolanyl-6-chloropurine (ACP), L-FDDC (5-fluoro-3'-thia-2',3'-dideoxycytidine), L-enantiomers of 3'-fluoro-modified β-2'-deoxyribonucleoside 5'-triphosphates, famciclovir, penciclovir, bis-Pom PMEA (adefovir, dipivoxil); lobucavir, ganciclovir, and ribavarin.

The active anti-HBV agents can also be administered in combination with antibiotics, other antiviral compounds, antifungal agents, or other pharmaceutical agents administered for the treatment of secondary infections.

Brief Description of the Figures

Figure 1 is an illustration of a general reaction scheme for the stereospecific synthesis of 3'-substituted β-L-dideoxynucleosides.

Figure 2 is an illustration of a general reaction scheme for the stereospecific synthesis of 2'-substituted β-L-dideoxynucleosides.

Figure 3 is an illustration of one process for the preparation of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine (L-3'-A-5-FddC).

Figure 4 is an illustration of one process for the preparation of β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine (L-2'-A-5-FddC).

Detailed Description of the Invention

As used herein, the term "substantially in the form of" or "substantially in the absence of" or "substantially free of" refers to a nucleoside composition that includes at least approximately 95%, and preferably approximately 97%, 98%, 99%, or 100% of a single enantiomer of that nucleoside.

The term alkyl, as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of C_1 to C_{10}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, cyclobutyl,

pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 1991. The term lower alkyl, as used herein, and unless otherwise specified, refers to a C_1 to C_4 ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, or t-butyl group.

As used herein, the term acyl specifically includes but is not limited to C(O)alkyl, C(O)aryl, acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic, or a the residue of an amino acid moiety.

The term aryl, as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The aryl group can be optionally substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 1991.

As used herein, the term amino acid includes natural and unnatural amino acids and includes but is not limited to alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaoyl, lysinyl, argininyl, and histidinyl.

A prodrug, as used herein, refers to a pharmaceutically acceptable derivative of the specifically disclosed nucleoside, that is converted into the nucleoside on administration in vivo, or that has activity in itself. Nonlimiting examples are the 5' and N^4-cytosine acylated or alkylated derivatives of the active compound, as well as the 5'-monophosphate, diphosphate, or triphosphate derivatives, other phosphates, or stablized nucleotide prodrugs, or 5'-ether lipids as described in more detail below. For example, the nucleoside is provided as the monophosphate, diphosphate or triphosphate in a formulation that protects the

compound from dephosphorylation. Formulations include liposomes, lipospheres, microspheres or nanospheres (of which the latter three can be targeted to infected cells).

The invention as disclosed herein is a method and composition for the treatment of HBV infection and other viruses replicating in a like manner, in humans or other host animals, that includes administering an effective HBV-treatment amount of one or more of the above-identified compounds, or a physiologically acceptable derivative, or a physiologically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier. The compounds of this invention either possess anti-HBV activity, or are metabolized to a compound or compounds that exhibit anti-HBV activity.

Structure and Preparation of Active Nucleosides

Stereochemistry

Since the 1' and 4' carbons of the sugar (referred to below generically as the sugar moiety) of the nucleosides are chiral, their nonhydrogen substituents (CH_2OR and the pyrimidine or purine base, respectively) can be either cis (on the same side) or trans (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the "primary" oxygen (that between the C1' and C4'-atoms is in back): "β" or "cis" (with both groups "up", which corresponds to the configuration of naturally occurring nucleosides, i.e., the D configuration), "β" or cis (with both groups "down", which is a nonnaturally occurring configuration, i.e., the L configuration), "α "or "trans" (with the C2 substituent "up" and the C5 substituent "down"), and "α " or trans (with the C2 substituent "down" and the C5 substituent "up").

The active nucleosides of the present invention are in the β-L-configuration, with the azido group in the ribosyl configuration.

Prodrug Formulations

The nucleosides disclosed herein can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent active compound, or that exhibits activity in itself. In one embodiment, the hydrogen of the 5'-OH group is replaced by a C_1-C_{20} alkyl; acyl including those in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C_1-C_{20} alkyl, phenyl, or benzyl; a naturally occurring or nonnaturally occurring amino acid; a 5'-ether lipid or a 5'-

phosphoether lipid; alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy; a dicarboxylic acid such as succinic acid; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; or a mono, di or triphosphate ester.

One or both hydrogens of the amino groups on the purine or pyrimidine base can be replaced by a C_1-C_{20} alkyl; acyl in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C_1-C_{20} alkyl, phenyl, or benzyl; alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy.

The active nucleoside can also be provided as a 5'-ether lipid, as disclosed in the following references: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.J., D. L.W., and C. Piantadosi. 1990. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res Hum Retroviruses. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991. Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity. J Med Chem. 34:1408.1414; Hostetler, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3,-deoxythymidine. Antimicrob Agents Chemother. 36:2025.2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, 1990. Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides. J. Biol Chem. 265:6112.7.

Stablized Nucleotides

Any of the nucleosides described herein can be administered as a nucleotide prodrug or phospholipid prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphoate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the the phosphate moiety are alkyl, aryl, steroids,

carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, **27** (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect. Nonlimiting examples of nucleotide prodrugs are described in the following references.

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Chemother. 1, 355-360; McGuigan, C., Nicholls, S.R., O'Connor, T.J., and Kinchington, D. (1990c) Synthesis of some novel dialkyl phosphate derivative of 3'-modified nucleosides as potential anti-AIDS drugs. *Antiviral Chem. Chemother.* 1, 25-33; McGuigan, C., Devine, K.G., O'Connor, T.J., and Kinchington, D.(1991) Synthesis and anti-HIV activity of some haloalky phosphoramidate derivatives of 3'-azido-3'deoxythylmidine (AZT); potent activity of the trichloroethyl methoxyalaninyl compound. *Antiviral Res.* 15, 255-263; McGuigan, C., Pathirana, R.N., Mahmood, N., Devine, K.G. and Hay, A.J. (1992) Aryl phosphate derivatives of AZT retain activity against HIV1 in cell lines which are resistant to the action of AZT. *Antiviral Res.* 17, 311-321; McGuigan, C., Pathirana, R.N., Choi, S.M., Kinchington, D. and O'Connor, T.J. (1993a) Phosphoramidate derivatives of AZT as inhibitors of HIV; studies on the carboxyl terminus. *Antiviral Chem. Chemother.* 4, 97-101; McGuigan, C., Pathirana, R.N., Balzarini, J. and De Clercq, E. (1993b) Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.* **36**, 1048-1052.

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Preparation of the Active Compounds

The nucleosides used in the disclosed method to treat HBV infections in a host organism can be prepared according to known methods. A general process for the stereospecific synthesis of 3'-substituted β-L-dideoxynucleosides is shown in Figure 1. A general process for the steroespecific synthesis of 2'-substituted β-L-dideoxynucleosides is shown in Figure 2. A detailed synthesis of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine is provided in Figure 3. A detailed synthesis of β-L-(2'-azido)-2',3'-dideoxy)-5-fluorocytosine is provide in Figure 4 and in Example 2 below.

Example 1

Preparation of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine

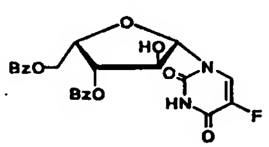
Melting points were determined in open capillary tubes on a Gallenkamp MFB-595-010 M apparatus and are uncorrected. The UV absorption spectra were recorded on an Uvikon 931 (KONTRON) spectrophotometer in ethanol. ^{1}H-NMR spectra were run at room temperature in DMSO-d_6 with a Bruker AC 250 or 400 spectrometer. Chemical shifts are given in ppm, DMSO-d_5 being set at 2.49 ppm as reference. Deuterium exchange, decoupling experiments or 2D-COSY were performed in order to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), m (multiplet). All J-values are in Hz. FAB mass spectra were recorded in the positive- (FAB>0) or negative (FAB<0) ion mode on a JEOL DX 300 mass spectrometer. The matrix was 3-nitrobenzyl alcohol (NBA) or a mixture (50:50, v/v) of glycerol and thioglycerol (GT). Specific rotations were measured on a Perkin-Elmer 241 spectropolarimeter (path length 1 cm) and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analysis were carried out by the "Service de Microanalyses du CNRS, Division de Vernaison" (France). Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values. Thin layer chromatography was performed on precoated aluminium sheets of Silica Gel 60 F_{254} (Merck, Art. 5554), visualisation of products being accomplished by UV absorbency followed by charring with 10% ethanolic sulfuric acid and heating. Column chromatography was carried out on Silica Gel 60 (Merck, Art. 9385) at atmospheric pressure.

1-(2-O-Acetyl-3,5-di-O-Benzoyl-β-L-Xylofuranosyl)-5-Fluorouracil (2)

A suspension of 5-fluorouracil (5.0 g, 38.4 mmol) was treated with hexamethyldisilazane (HMDS, 260 mL) and a catalytic amount of ammonium sulfate during 18 h under reflux. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue obtained as a colourless oil was diluted with anhydrous 1,2-dichloroethane (260 mL). To the resulting solution was added 1,2-di-*O*-acetyl-3,5-di-*o*-benzoyl-L-xylofuranose 1 (11.3 g, 25.6 mmol) [Ref.: Gosselin, G.; Bergogne, M.-C.; Imbach, J.-L., "Synthesis and Antiviral Evaluation of β-L-Xylofuranosyl Nucleosides of the Five Naturally Occuring Nucleic Acid Bases", *Journal of Heterocyclic Chemistry,* 1993, 30 (Oct.-Nov.), 1229-1233] in anhydrous 1,2-dichloroethane (130 mL), followed by addition of trimethylsilyl

trifluoromethanesulfonate (TMSTf, 9.3 mL, 51.15 mmol). The solution was stirred for 6 h at room temperature under argon atmosphere, then diluted with chloroform (1 L), washed with the same volume of a saturated aqueous sodium hydrogen carbonate solution and finally with water (2× 800 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting crude material was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-4%) in methylene chloride] to give 2 (11.0 g, 84% yield) as a white foam; mp = 96-98°C; UV (ethanol): λ_{max} = 228 nm (ε = 25900) 266 nm (ε = 9000), λ_{min} = 250 nm (ε = 7200); ^{1}H-NMR (DMSO-d_6): δ 11.1 (br s, 1H, NH), 8.05 (1H, H-6, $J_{6\text{-}F5}$ = 6.8 Hz), 7.9-7.4 (m, 10H, 2 C_6H_5CO), 5.99 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 3.1 Hz), 5.74 (dd, 1H, H-3', $J_{3'\text{-}2'}$ = 4.2 Hz and $J_{3'\text{-}4'}$ = 2.3 Hz), 5.54 (t, 1H, H-2', $J_{2'\text{-}1'} = J_{2'\text{-}3'}$ = 2,9 Hz), 4.8-4.6 (m, 3H, H-4', H-5' and H-5"); MS: FAB>0 (matrix GT) *m/z* 513 $(M+H)^+$, 383 $(S)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 511 $(M-H)^-$, 469 $(M-CH_3CO)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -91 (c, 0.88 DMSO); Anal $C_{25}H_{21}FN_2O_9$ (C, H, N, F).

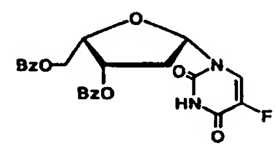
1-(3,5-Di-O-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 3



Hydrazine hydrate (2.80 mL, 57.4 mmol) was added to a solution of 1-(2-*O*-acetyl-3,5-di-*o*-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 2 (9.80 g, 19.1 mmol) in acetic acid (35 mL) and pyridine (150 mL). The resulting solution was stirred overnight at room temperature. Acetone (50 mL) was added and the mixture was stirred during 2 h. The reaction mixture was concentrated to a small volume and partitioned between ethyl acetate (200 mL) and water (200 mL). Layers were separated and the organic phase was washed with a saturated aqueous sodium hydrogen carbonate solution (2× 200 mL), and finally with water (2× 200 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-5%) in methylene chloride] to give pure 3 (7.82 g, 87%), which was crystallized from methylene chloride; mp = 93-97°C; UV (ethanol): λ_{max} = 227 nm

(ε = 22800) 267 nm (ε = 8200), λ_{min} = 249 nm (ε =5900); ^{1}H-NMR (DMSO-d_6): δ 11.9 (br s, 1H, NH), 8.06 (d, 1H, H-6, $J_{6\text{-}F5}$ = 6.9 Hz), 8.0-7.4 (m, 10H, 2 C_6H_5CO), 6.35 (d, 1H, OH-2', $J_{OH\text{-}2'}$ = 3.8 Hz), 5.77 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 3.3 Hz), 5.43 (dd, 1H, H-3', $J_{3'\text{-}2'}$ = 3.1 Hz and $J_{3'\text{-}4'}$ = 1.9 Hz) 4.8-4.6 (m, 3H, H-4', H-5' and H-5"), 4.43 (t, 1H, H-2', J = 2.3 Hz); MS: FAB>0 (matrix GT) *m/z* 941 $(2M+H)^+$, 471 $(M+H)^+$, 341 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 939 $(2M\text{-}H)^-$, 469 $(M\text{-}H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -110 (c, 1.55 DMSO).

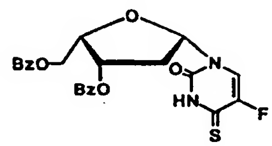
1-(2-Deoxy-3,5-di-o-benzoyl-β-L-*threo*-pentofuranosyl)-5-fluorouracil 5



To a solution of 1-(3,5-di-*O*-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 3 (15.4 g, 32.7 mmol) in anhydrous acetonitrile (650 mL) were added *O*-phenyl chlorothionoformate (6.80 mL, 49.1 mmol) and 4-dimethylaminopyridine (DMAP, 12.0 g, 98.2 mmol). The resulting solution was stirred at room temperature under argon during 1 h and then evaporated under reduced pressure. The residue was dissolved in methylene chloride (350 mL) and the organic solution was successively washed with water (2× 250 mL), with an ice-cold 0.5 N hydrochloric acid (250 mL) and with water (2× 250 mL), dried over sodium sulphate and evaporated under reduced pressure. The crude material 4 was co-evaporated several times with anhydrous dioxane and dissolved in this solvent (265 mL). To the resulting solution were added under argon tris(trimethylsilyl)silane hydride (12,1 mL, 39.3 mmol) and α,α'-azoisobutyronitrile (AIBN, 1.74 g, 10.8 mmol). The reaction mixture was heated and stirred at 100°C for 2.5 h under argon, then cooled to room temperature and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in chloroform] to give pure 5 (13.0 g, 87%), which was crystallized from a diethyl ether/methanol mixture; mp = 182-184°C; UV (ethanol): λ_{max} = 229 nm (ε = 25800), 269 nm (ε = 9300), λ_{min} = 251 nm (ε = 6500); ^{1}H-NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.05 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 8.0-7.4 (m, 10H, 2 C_6H_5CO), 6.15 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 7.4 Hz), 5.68 (t, 1H, H-3', $J_{3'\text{-}2'}$ = $J_{3'\text{-}4'}$ = 4.2 Hz), 4.8-4.6 (m, 2H, H-5' and H"-5), 4.6 (m, 1H, H-4'), 3.0-2.8 (m, 1H, H-2'), 2.5-2.3 (d, 1H, H-2", J = 14.8 Hz); MS:

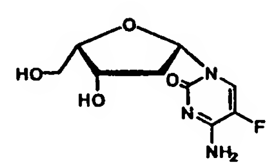
FAB>0 (matrix GT) *m/z* 455 $(M+H)^+$, 325 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 452 $(M-H)^-$, 129 $(B)^-$; $[\alpha]_D^{20}$ = -125 (c 1.05 DMSO); Anal $C_{23}H_{19}FN_2O_7$ (C, H, N, F).

1-(2-Deoxy-3,5-di-o-benzoyl-β-L-threo-pentofuranosyl)-4-thio-5-fluorouracil 6



Lawesson's reagent (3.1 g, 7.70 mmol) was added under argon to a solution of 5 (5.0 g, 11.0 mmol) in anhydrous 1,2-dichloroethane (200 mL) and the reaction mixture was stirred overnight under reflux. The solvent was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in chloroform] to give the 4-thio intermediate 6 (80% yield) as a yellow foam; mp = 178-179°C; UV (ethanol): λ_{max} = 230 nm (ε = 24900), 273 nm (ε = 6900), 333 nm (ε = 19200), λ_{min} = 258 nm (ε = 5900), 289 nm (ε = 5300); ^{1}H-NMR (DMSO-d_6): δ 13.1 (br s, 1H, NH), 8.10 (d, 1H, H-6, $J_{6\text{-}F5}$ = 4,6 Hz), 8.1-7.4 (m, 10H, 2 C_6H_5CO), 6.09 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 7.3 Hz), 5.68 (t, 1H, H-3', $J_{3'\text{-}2'} = J_{3'\text{-}4'}$ = 4.1 Hz), 4.9-4.8 (m, 2H, H-5' and H-5"), 4.7 (m, 1H, H-4'), 2.9 (m, 1H, H-2'), 2.5 (m, 1H, H-2"); MS: FAB>0 (matrix GT) *m/z* 941 $(2M+H)^+$, 471 $(M+H)^+$, 325 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 469 $(M-H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -271 (c, 0,90 DMSO); Anal $C_{23}H_{19}FN_2O_6S$ (C, H, N, F).

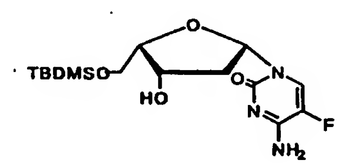
1-(2-Deoxy-β-L-*threo*-pento furanosyl)-5-fluorocytosine 7



A solution of this 4-thio intermediate 6 (1.0 g, 2.13 mmol) in methanolic ammonia (previously saturated at -10°C and tightly stopped) (60 mL) was heated at 100°C in a

stainless-steel bomb for 3 h and then cooled to 0°C. The solution was evaporated to dryness under reduced pressure and the residue co-evaporated several times with methanol. The crude material was dissolved in water and the resulting solution was washed four times with methylene chloride. The aqueous layer was evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (3-20%) in methylene chloride]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0,45 µm, Millipore) to provide 0.44 g of 7 (84% yield) which was crystallized from an ethyl acetate/methanol mixture; mp = 199-201°C; UV (ethanol): λ_{max} = 226 nm (ε = 7700), 281 nm (ε = 8500), λ_{min} = 262 nm (ε = 6300); ^{1}H-NMR (DMSO-d_6): δ 7.99 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.4 Hz), 7.7-7.4 (br d, 2H, NH_2), 5.98 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 8.1 Hz), 5.25 (d, 1H, OH-3', $J_{OH\text{-}3'}$ = 3.4 Hz), 4.71 (t, 1H, OH-5', $J_{OH\text{-}5'}$ = $J_{OH\text{-}5''}$ = 5.6 Hz), 4.2 (m, 1H, H-3'), 3.8-3.6 (m, 3H, H-4', H-5' and H-5''), 2.5 (m, 1H, H-2'), 1.8 (m, 1H, H-2''); MS: FAB>0 (matrix GT) *m/z* 491 $(2M+H)^+$, 246 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 (matrix GT) *m/z* 489 $(2M-H)^-$, 244 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -21 (c, 0.92 DMSO); Anal $C_9H_{12}FN_3O_4$ (C, H, N, F).

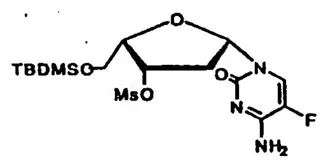
1-(2-Deoxy-5-O-*t*-butyldimethyl silyl-β-L-*threo*-pentofuranosyl)-5-fluorocytosine 8



To a solution of 7 (1.69 g, 6.89 mmol) in dry pyridine (35 mL) was added dropwise under argon atmosphere *t*-butyldimethylsilyl chloride (1.35 g, 8.96 mmol) and the mixture was stirred for 5 h at room temperature. Then the mixture was poured onto a saturated aqueous sodium hydrogen carbonate solution (100 mL) and extracted with chloroform (3× 150 mL). Combined extracts were washed with water (2× 200 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (2-10%) in methylene chloride] to give pure 8 (2.94 g, 87%), as a white solid: mp 177-179°C; UV (ethanol): λ_{max} 241 nm (ε 9900), 282 nm (ε 10000), λ_{min} 226 nm (ε 8200), 263 nm (ε 7600); ^{1}H NMR (DMSO-d_6): δ 7.95 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.3 Hz), 7.8-7.3 (br d, 2H, NH_2), 6.00 (dd, 1H, H-1',

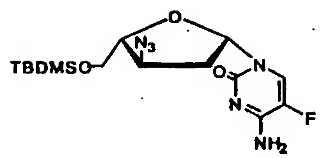
$J_{1'-2'}$ = 6.1 Hz and $J_{1'-2''}$ = 1.9 Hz), 5.3 (br s, 1H, OH-3'), 4.2 (br s, 1H, H-3'), 3.9-3.7 (m, 3H, H-4', H-5' and H-5"), 2.5 (m, 1H, H-2'), 1.81 (br d, 1H, H-2", J = 14.6 Hz), 0.86 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 719 $(2M+H)^+$, 360 $(M+H)^+$, 130 $(BH_2)^+$, 115 $(TBDMS)^+$; FAB<0 *m/z* 717 $(2M-H)^-$, 358 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -23 (c, 0.96 DMSO).

1-(2-Deoxy-3-O-mesyl-5-O-*t*-butyl dimethylsilyl-β-L-*threo*-pento furanosyl)-5-fluorocytosine 9



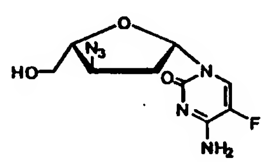
A suspension of 8 (0.70 g, 1.96 mmol) in dry pyridine (30 mL) was stirred under argon and cooled to 0°C. Methanesulfonyl chloride (MsCl, 0.46 mL, 5.88 mmol) was added dropwise and the reaction mixture stirred at 0°C for 5 h. Then the mixture was poured onto ice/water (100 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with a 5% aqueous sodium hydrogen carbonate solution (100 mL), with water (2× 100 mL), dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (8-12%) in toluene] to give pure 9 (0.56 g, 65%) as a white solid: mp 83-84 °C; UV (ethanol): λ_{max} 242 nm (ε 8500), 282 nm (ε 8800), λ_{min} 225 nm (ε 6400), 264 nm (ε 6300); ^1H NMR (DMSO-d_6): δ 7.8-7.3 (br d, 2H, NH_2), 7.60 (d, 1H, H-6, J_{6-F5} = 7.0 Hz), 5.93 (dd, 1H, H-1', $J_{1'-2'}$ = 4.5 Hz and $J_{1'-2''}$ = 2.0 Hz), 5.2 (m, 1H, H-3'), 4.1 (m, 1H, H-4'), 3.9-3.7 (m, 2H, H-5' and H-5"), 3.17 (s, 3H, CH_3SO_2), 2.7 (m, 1H, H-2'), 2.1 (m, 1H, H-2"), 0.99 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 875 $(2M+H)^+$, 438 $(M+H)^+$, 342 $(M-CH_3SO_3)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 873 $(2M-H)^-$, 436 $(M-H)^-$, 128 $(B)^-$, 95 $(CH_3SO_3)^-$; $[\alpha]_D^{20}$ = -28 (c, 0.96 DMSO).

1-(2,3-Dideoxy-3-azido-5-O-*t*-butyl dimethylsilyl-β-L-*erythro*-pento furanosyl)-5-fluorocytosine 10



To a solution of 9 (520 mg, 1.19 mmol) in anhydrous dimethylformamide (12 mL) was added lithium azide moistened with 10% methanol (300 mg, 5.31 mmol). The reaction mixture was stirred at 100°C during 2.5 h, and then cooled to room temperature, poured onto ice/water (200 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with saturated aqueous sodium hydrogen carbonate solution (2× 100 mL), with water (5× 100 mL), and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : methanol (4%) in chloroform] to give pure 10 (327 mg, 72%), which was crystallized from a diethyl ether/methanol mixture: mp 146-147°C; UV (ethanol): λ_{max} 243 nm (ε 8700), 283 nm (ε 8400), λ_{min} 226 nm (ε 7200), 264 nm (ε 6700) ; ^{1}H NMR (DMSO-d_6): δ 7.90 (d, 1H, H-6, J_{6-F5} = 7.0 Hz), 7.8-7.5 (br d, 2H, NH_2), 6.0 (m, 1H, H-1'), 4.3 (m, 1H, H-3'), 3.9-3.7 (m, 3H, H-4', H-5' and H"-5), 2.4-2.2 (m, 2H, H-2' and H-2"), 0.87 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 769 $(2M+H)^+$, 385 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 383 $(M-H)^-$; $[\alpha]_D^{20}$ = -67 (c, 0.96 DMSO).

1-(2,3-Dideoxy-3-azido-β-L-*erythro*-pentofuranosyl)-5-fluorocytosine 11 (3'-N_3-β-L-5-FddC)



A 1 M solution of tetrabutylammonium trifluoride in tetrahydrofurane (TBAF/THF, 1.53 mL, 1.53 mmol) was added to a solution of 10 (295 mg, 0.67 mmol) in anhydrous THF (4 mL). The resulting mixture was stirred at room temperature for 1.5 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (4-8%) in chloroform]. Finally, the appropriate fractions were

evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0,45 µm, Millipore) to give pure 11 (199 mg, 96%), which was crystallized from ethanol: mp 188-189°C (lit.: mp 164-166°C for the D-enantiomer); UV (ethanol): λmax 243 nm (ε 8700), 283 nm (ε 8100), λmin 226 nm (ε 7100), 264 nm (ε 6500) ; ^{1}H NMR (DMSO-d_6): δ 8.08 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.3 Hz), 7.8-7.5 (br d, 2H, NH_2), 6.0 (m, 1H, H-1'), 5.3 (br s, 1H, OH-5'), 4.4 (m, 1H, H-3'), 3.8 (m, 1H, H-4'), 3.7-3.5 (m, 2H, H-5' and H-5"), 2.3 (m, 2H, H-2' and H-2"); MS (matrix GT): FAB>0 *m/z* 811 $(3M+H)^+$, 725 $(2M+2G+H)^+$, 633 $(2M+G+H)^+$, 541 $(2M+H)^+$, 363 $(M+G+H)^+$, 271 $(M+H)^+$, 142 $(S)^+$, 130 $(BH_2)^+$; FAB<0*m/z* 647 $(2M+T-H)^-$, 539 $(2M-H)^-$, 377 $(M+T-H)^-$, 269 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -31 (c, 0.90 DMSO); Anal. ($C_9H_{11}FN_6O_3$) C, H, N, F.

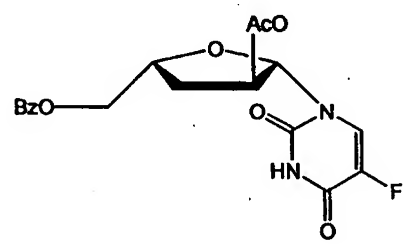
Analytical data

Compd	Formula	Anal Calculated				Anal Found			
		C	H	N	F	C	H	N	F
2	$C_{25}H_{21}FN_2O_9$	58.59	4.13	5.47	3.71	58.33	4.25	4.24	3.49
5	$C_{23}H_{19}FN_2O_7$	60.79	4.21	6.17	4.18	61.22	4.26	6.18	3.90
6	$C_{23}H_{19}FN_2O_6S$	58.71	4.07	5.96	4.04	58.25	4.10	5.91	4.00
7	$C_9H_{12}FN_3O_4$	44.08	4.87	17.17	7.75	43.87	5.13	16.81	7.42
11	$C_9H_{11}FN_6O_3$	40.00	4.10	31.10	7.03	40.35	3.83	31.38	7.12

Example 2 Preparation of β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine

General procedures and instrumientation used have been described in Example 1 in the Experimental protocols part of the synthesis of the 3' isomer (3'-N_3-β-L-FddC).

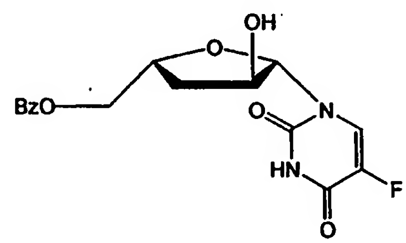
1-(2-O-Acetyl-3-deoxy-5-O-benzoyl-β-L-erythro-pentofuranosyl)-5-fluorouracil 13



A suspension of 5-fluorouracil (5.15 g, 39.6 mmol) was treated with hexamethyldisilazane (HMDS, 257 mL) and a catalytic amount of ammonium sulfate during 18 h under reflux. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue obtained as a colourless oil was diluted with anhydrous 1,2-dichloroethane (290 mL). To the resulting solution was added 1,2-di-*O*-acetyl-3-deoxy-5-*O*-benzoyl-L-*erythro*-pentofuranose 12 (8.5 g, 26.4 mmol) [Ref.: Mathé, C., *Ph.D. Dissertation*, Université de Montpellier II -Sciences et Techniques du Languedoc, Montpellier (France), September 13, 1994; Gosselin, G.; Mathé, C.; Bergogne, M.-C.; Aubertin, A.M.; Kirn, A.; Sommadossi, J.P.; Schinazi, R.F.; Imbach, J.L., "2'- and/or 3'-deoxy-β-L-pentofuranosyl nucleoside derivatives: stereospecific synthesis and antiviral activities," *Nucleosides & Nucleotides*, 1994, 14 (3-5), 611-617] in anhydrous 1,2-dichloroethane (120 mL), followed by addition of trimethylsilyl trifluoromethanesulfonate (TMSTf, 9.6 mL, 52.8 mmol). The solution was stirred for 5 h at room temperature under argon atmosphere, then diluted with chloroform (200 mL), washed with the same volume of a saturated aqueous sodium hydrogen carbonate solution and finally with water (2× 300 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting crude material was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-6%) in methylene chloride] to give pure 13 (8.59 g, 83%), which was crystallized from toluene: mp 65-68°C; UV (ethanol): λ_{max} 228 nm (ε 11200) 268 nm (ε 14000), λ_{min} 242 nm (ε 7800); ^{1}H NMR (DMSO-d_6): δ 11.9 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.8 (m, 1H,

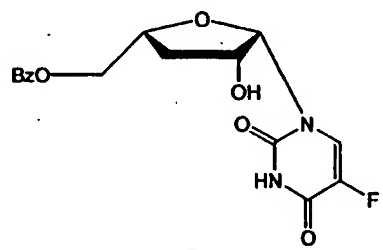
H-1'), 5.3 (m, 1H, H-2'), 4.6-4.5 (m, 3H, H-4', H-5' and H-5"), 2.4-2.3 (m, 1H, H-3'), 2.1-2.0 (m, 4H, H-3" and CH_3CO); MS (matrix GT): FAB>0 *m/z* 393 $(M+H)^+$, 263 $(S)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 391 $(M-H)^-$, 331 $(M-[CH_3CO_2H]-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20} = -8$ (*c*,1.00 DMSO); Anal. ($C_{18}H_{17}FN_2O_7$; $^2/_3$ C_7H_8) C, H, N, F.

1-(3-Deoxy-5-O-benzoyl-β-L-erythro-pentofuranosyl)-5-fluorouracil 14



To a solution of 13 (5.90 g, 15.0 mmol) in tetrahydrofurane (THF, 175 mL), was added sodium methoxide (2.84 g, 52.6 mmol). The resulting suspension was stirred at room temperature during 5 h and then neutralized by addition of Dowex 50 W X 2 (H^+ form). The resin was filtered and washed with warm methanol, and the combined filtrates were evaporated to dryness. Column chromatography of the residue on silica gel [eluent: stepwise gradient of methanol (0-8%) in methylene chloride] afforded 14 (4.11 g, 78%), which was crystallized from a methylene chloride/methanol mixture: mp 154-156°C; UV (ethanol): λ_{max} 226 nm (ε 23000), 268 nm (ε 16000), λ_{min} 246 nm (ε 8900); ^1H NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.6 (br s, 2H, H-1' and OH-2'), 4.5 (m, 3H, H-4', H-5' and H-5"), 4.3 (m, 1H, H-2'), 2.1-2.0 (m, 1H, H-3'), 1.9 (m, 1H, H-3"); MS (matrix GT): FAB>0 *m/z* 701 $(2M+H)^+$, 351 $(M+H)^+$, 221 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 1049 $(3M-H)^-$, 699 $(2M-H)^-$, 441 $(M+G-H)^-$, 349 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20} = -3$ (*c*, 1.04 DMSO); Anal. ($C_{16}H_{15}FN_2O_6$) C, H, N, F.

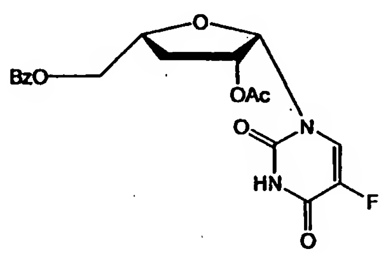
1-(3-Deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-5-fluorouracil 15



Dicyclohexylcarbodiimide (DCC, 3.53 g, 17.1 mmol) and dichloroacetic acid (0.235 mL, 2.56 mmol) were added to a solution of 14 (2.00 g, 5.71 mmol) in anhydrous benzene (50 mL), DMSO (35 mL) and pyridine (0.46 mL). The resulting solution was stirred at room temperature under argon during 4 h and diluted with ethyl acetate (300 mL). oxalic acid (1.54 g, 17.1 mmol) dissolved in methanol (4.6 mL) was added and the reaction mixture was stirred at room temperature during 1 h and then filtered to eliminate precipitated dicyclohexylurea (DCU). The filtrate was washed with brine (3× 300 mL), with a saturated aqueous sodium hydrogen carbonate solution (2× 300 mL) and finally with water (3× 200 mL) before being dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was co-evaporated several times with absolute ethanol and dissolved in a mixture of absolute ethanol (31 mL) and anhydrous benzene (15 mL). The resulting solution was then cooled to 0°C and sodium borohydride ($NaBH_4$, 0.32 g, 8.56 mmol) was added. The reaction mixture was stirred at room temperature under argon during 1 h and diluted with ethyl acetate (300 mL) filtered. The filtrate was washed with a saturated aqueous sodium chloride solution (3× 300 mL) and with water (2× 200 mL) before being dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-6%) in chloroform] to give pure 15 (1.10 g, 55%), as a white foam: mp 171-172°C; UV (ethanol): λ_{max} 228 nm (ε 14700) 270 nm (ε 9100), λ_{min} 248 nm (ε 5000); ^{1}H NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.90 (dd, 1H, H-1', $J_{1'-2'}$ = 4.1 Hz and $J_{1'-F5}$ = 1.8 Hz), 5,5 (br s, 1H, OH-2'), 4.7 (br q, 1H, H-4', J = 11.7 Hz and J = 7.0 Hz), 4.4-4.3 (m, 3H, H-2', H-5' and H-5"), 2.4 (m, 1H, H-3'), 1.9-1.8 (m, 1H, H-3"); MS (matrix GT): FAB>0 *m/z* 701

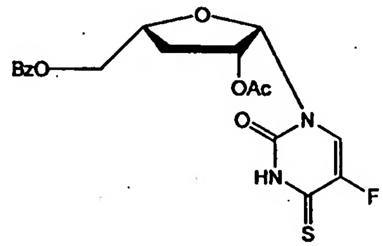
$(2M+H)^+$, 351 $(M+H)^+$, 221 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 1049 $(3M-H)^-$, 699 $(2M-H)^-$, 349 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -101 (*c*, 0.70 DMSO)

1-(2-O-Acetyl-3-deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-5-fluorouracil 16



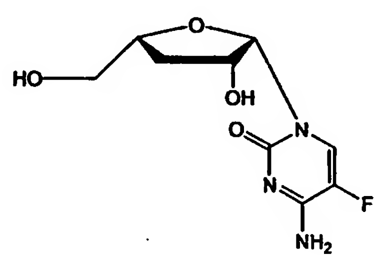
Acetic anhydride (0.88 mL, 9.28 mmol) was added under argon to a solution of 15 (2.50 g, 7.14 mmol) in dry pyridine (50 mL) and the resulting mixture was stirred at room temperature for 22 h. Then, ethanol was added and the solvents were evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in methylene chloride] to give pure **16** (2.69 g, 96%) as a white foam; mp = 68-70°C (foam); UV (ethanol) : λ_{max}= 239 nm (ε= 15000) 267 nm (ε= 8800), λ_{min}= 248 nm (ε= 5600) ; ^{1}H NMR (DMSO-d_6) : δ ppm 11.9 (br s, 1H, NH), 8.1-7.5 (m, 6H, C_6H_5CO and H-6), 6.10 (d, 1H, H-1', $J_{1'-2'}$= 4.3 Hz), 5.4 (m, 1H, H-2'), 4.6-4.4 (m, 3H, H-4', H-5' and H-5"), 2.6 (m, 1H, H-3'), 2.03 (m, 1H, H-3"), 1,86 (s, 3H, CH_3CO) ; MS (matrix GT): FAB>0 *m/z* 785 $(2M+H)^+$, 393 $(M+H)^+$, 263 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$, 43 $(CH_3CO)^+$; FAB<0 *m/z* 391 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$, 59 $(CH_3CO_2)^-$; $[\alpha]_D^{20}$= -81 (*c*, 0.95 DMSO).

1-(2-O-Acetyl-3-deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-4-thio-5-fluorouracil 17



Lawesson's reagent (1.9 g, 4.69 mmol) was added under argon to a solution of 16 (2.63 g, 6.70 mmol) in anhydrous 1,2-dichloroethane (165 mL) and the reaction mixture was stirred overnight under reflux. The solvent was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-3%) in methylene chloride] to give the 4-thio derivative 17 (2.65 g, 96% yield) as a yellow foam; mp = 78-79°C (foam) ; UV (ethanol) : λ_{max}= 230 nm (ε= 15900) 334 nm (ε= 15600), λ_{min}= 288 nm (ε= 3200) ; ^{1}H NMR (DMSO-d_6) : δ ppm 13.2 (br s, 1H, NH), 8.1-7.5 (m, 6H, C_6H_5CO and H-6), 6.08 (d, 1H, H-1', $J_{1'-2'}$= 4.3 Hz), 5.4 (m, 1H, H-2'), 4.7-4.4 (m, 3H, H-4', H-5' and H-5"), 2.6 (m, 1H, H-3'), 2.0 (m, 1H, H-3"), 1.84 (s, 3H, CH_3CO) ; MS (matrix GT): FAB>0 *m/z* 409 $(M+H)^+$, 263 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$, 43 $(CH_3CO)^+$; FAB<0 *m/z* 407 $(M-H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$, 59 $(CH_3CO_2)^-$; $[\alpha]_D^{20}$= -155 (*c*, 1.00 DMSO).

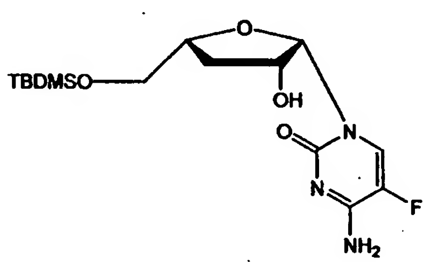
1-(3-Deoxy-β-L-threo-pentofuranosyl)-5-fluorocytosine 18



A solution of the 4-thio derivative 17 (0.86 g, 2.19 mmol) in methanolic ammonia (previously saturated at -10°C and tightly stopped) (44 mL) was heated at 100°C in a

stainless-steel bomb for 3 h and then cooled to 0°C. The solution was evaporated to dryness under reduced pressure and the residue co-evaporated several times with methanol. The crude material was dissolved in water and the resulting solution was washed four times with methylene chloride. The aqueous layer was evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (3-12%) in chloroform]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0.45 μm, Millipore) to provide 0.46 g of 18 (86% yield) which was crystallized from a methylene/methanol mixture; mp = 137-138°C ; UV (ethanol) : λ_{max}= 240 nm (ε= 8300) 284 nm (ε= 8100), λ_{min}= 226 nm (ε= 7300) 263 nm (ε= 5500) ; ^{1}H NMR (DMSO-d_6) : δ ppm 8.34 (d, 1H, H-6, $J_{6\text{-}F5}$= 7.5 Hz), 7.7-7.4 (br pd, 2H, NH_2), 5.83 (dd, 1H, H-1', $J_{1'\text{-}2'}$= 4.4 Hz, $J_{1'\text{-}F5}$= 1.9 Hz), 5.22 (d, 1H, OH-2', $J_{OH\text{-}2'}$= 5.1 Hz), 5.15 (t, 1H, OH-5', $J_{OH\text{-}5'}$= $J_{OH\text{-}5''}$= 4.8 Hz), 4.3 (m, 1H, H-2'), 4.0 (m, 1H, H-4'), 3.6-3.5 (m, 2H, H-5' and H-5") 2.2 (m, 1H, H-3'), 1.7 (m, 1H, H-3") ; MS (matrix GT): FAB>0 *m/z* 491 $(2M+H)^+$, 246 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 244 $(M\text{-}H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$= -135 (*c*, 0.89 DMSO). Elemental analysis, $C_9H_{12}FN_3O_4$, ½ H_2O; Calc. C= 42.52 ; H= 5.15 ; N= 16.53 ; F= 7.47; Found: C= 43.16 ; H= 5.32 ; N= 16.97 ; F= 6.92

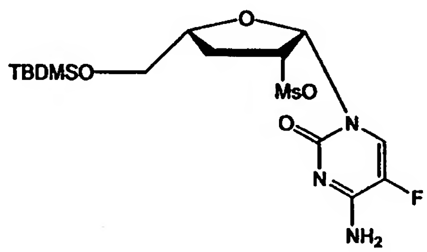
1-(3-Deoxy-5-O-t-butyldimethylsilyl-β-L-threo-pentofuranosyl)-5-fluorocytosine 19



To a solution of 18 (1.38 g, 5.63 mmol) in dry pyridine (30 mL) was added dropwise under argon atmosphere *t*-butyldimethylsilyl chloride (1.10 g, 7.32 mmol) and the mixture was stirred for 10 h at room temperature. Then the mixture was poured onto a saturated aqueous sodium hydrogen carbonate solution (100 mL) and extracted with chloroform (3× 150 mL). Combined extracts were washed with water (2× 200 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (2-10%) in methylene

chloride] to give pure 19 (1.74 g, 86% yield) as a white solid: mp 202-204°C; UV (ethanol): λ_{max} 241 nm (ε 7800), 284 nm (ε 7800), λ_{min} 226 nm (ε 6600), 263 nm (ε 5400) ; ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.1 Hz), 7.7-7.3 (br d, 2H, NH_2), 6.88 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 4.9 Hz and $J_{1'\text{-}F5}$ = 1.9 Hz), 5.24 (d, 1H, OH-3', $J_{OH\text{-}3'}$ = 4.6 Hz), 4.4 (m, 1H, H-2'), 4.0 (m, 1H, H-4'), 3.8-3.7 (m, 2H, H-5' and H-5"), 2.2 (m, 1H, H-3'), 1.7 (m, 1H, H-3"), 0.84 (s, 9H, $(CH_3)_3C$-Si), 0.06 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 1437 $(4M+H)^+$, 1078 $(3M+H)^+$, 719 $(2M+H)^+$, 360 $(M+H)^+$, 231 $(S)^+$, 130 $(BH_2)^+$, 115 $(TBDMS)^+$; FAB<0 *m/z* 1076 $(3M-H)^-$, 717 $(2M-H)^-$, 358 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -107 (*c*, 0.88 DMSO).

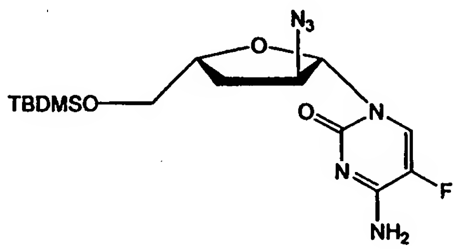
1-(3-Deoxy-2-O-mesyl-5-O-t-butyl-dimethylsilyl-β-L-threo-pentofuranosyl)-5-fluorocytosine 20



A suspension of 19 (1.70 g, 4.73 mmol) in dry pyridine (80 mL) was stirred under argon and cooled to 0°C. Methanesulfonyl chloride (MsCl, 1.21 mL, 15.6 mmol) was added dropwise and the reaction mixture stirred at 0°C for 5 h. Then the mixture was poured onto ice/water (300 mL) and extracted with chloroform (3× 300 mL). Combined extracts were washed with a 5% aqueous sodium hydrogen carbonate solution (300 mL), with water (2× 300 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (8-12%) in toluene] to give pure 20 (1.41 g, 68% yield) as a white solid: mp 75-76 °C; UV (ethanol): λ_{max} 243 nm (ε 8100), 282 nm (ε 7300), λ_{min} 225 nm (ε 6000), 265 nm (ε 6000); ^{1}H NMR (DMSO-d_6): δ 7.9-7.6 (br d, 2H, NH_2), 7.85 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 6.08 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 5.2 Hz and $J_{1'\text{-}F5}$ = 1.6 Hz), 5.4 (m, 1H, H-2'), 4.1 (m, 1H, H-4'), 3.9 (m, 1H, H-5'), 3.7 (m, 1H, H-5"), 3.11 (s, 3H, CH_3SO_2), 2.47 (m, 1H, H-3'), 2.0 (m, 1H, H-2"), 0.85 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 1312 $(3M+H)^+$, 875 $(2M+H)^+$, 438 $(M+H)^+$, 309 $(S)^+$, 130 $(BH_2)^+$; FAB<0 *m/z*

1310 (2M-H)⁻, 873 (2M-H)⁻, 436 (M-H)⁻, 128 (B)⁻, 95 (CH_3SO_3)⁻; $[\alpha]_D^{20}$ = -84 (*c*, 0.84 DMSO).

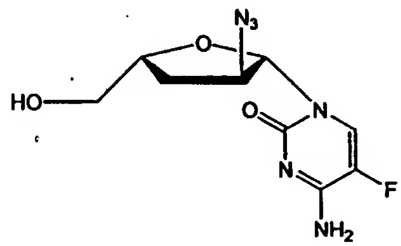
1-(2,3-Dideoxy-2-azido-5-O-t-butyldimethylsilyl-β-L-erythro-pentofuranosyl)-5-fluorocytosine 21



To a solution of 20 (442 mg, 1.01 mmol) in anhydrous dilmethylformamide (12 mL) was added lithium azide moistened with 10% methanol (265 mg, 4.87 mmol). The reaction mixture was stirred at 100°C during 2.5 h, and then cooled to room temperature, poured onto ice/water (200 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with a saturated aqueous sodium hydrogen carbonate solution (2× 100 mL), with water (5× 100 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : methanol (4%) in chloroform] to give pure 21 (291 mg, 75% yield) as a white solid: mp 147-148°C ; UV (ethanol): λ_{max} 242 nm (ε 7700), 283 nm (ε 7400), λ_{min} 226 nm (ε 6600), 264 nm (ε 5800); ^{1}H NMR (DMSO-d_6): δ 8.05 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 7.9-7.4 (br d, 2H, NH_2), 5.7 (br s, 1H, H-1'), 4.37 (d, 1H, H-2', $J_{2'\text{-}3'}$ = 5.5 Hz), 4.3 (m, 1H, H-4'), 4. (m, 1H, H-5'), 3.7 (m, 1H,H-5"), 2.0 (m, 1H, H-3'), 1.8 (m, 1H, H-3"), 0.88 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 769 $(2M+H)^+$, 385 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 1151 (3M-H)⁻, 767 (2M-H)⁻, 383 (M-H)⁻, 128 (B)⁻; $[\alpha]_D^{20}$ = +25 (*c*, 0.95 DMSO).

1-(2,3-Dideoxy-2-azido-β-L-erythro-pentofuranosyl)-5-fluorocytosine 22

(2'-N_3-β-L-5-FddC)



A 1 M solution of tetrabutylammonium trifluoride in tetrahydrofurane (TBAF/THF, 1.90 mL, 1.90 mmol) was added to a solution of 21 (480 mg, 1.25 mmol) in anhydrous THF (8 mL). The resulting mixture was stirred at room temperature for 1.5 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (4-8%) in chloroform]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0.45 μm, Millipore) to give pure 22 (304 mg, 90% yield), which was crystallized from ethanol: mp 219-221°C; UV (ethanol): λ_{max} 241 nm (ε 7700), 284 nm (ε 7300), λ_{min} 225 nm (ε 6500), 263 nm (ε 5400); ^{1}H NMR (DMSO-d_6): δ 8.31 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.4 Hz), 7.9-7.4 (br d, 2H, NH_2), 5.65 (m, 1H, H-1'), 5.32 (br s, 1H, OH-5'), 4.35 (d, 1H, H-2', $J_{2'\text{-}3'}$ = 5.6 Hz), 4.2 (m, 1H, H-4'), 3.8 (m, 1H, H-5'), 3.6 (m, 1H, H-5"), 2.1 (m, 1H, H-3'), 1.8 (m, 1H, H-2"); MS (matrix GT): FAB>0 *m/z* 541 $(2M+H)^+$, 363 $(M+G+H)^+$, 271 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 539 $(2M-H)^-$, 269 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = +29 (*c*, 0.85 DMSO); Anal. ($C_9H_{11}FN_6O_3$) C, H, N, F.

Analytical data

Compd	Formula	Anal. calculated				Anal. found			
		C	H	N	F	C	H	N	F
13	$C_{18}H_{17}FN_2O_7$, $^2/_3$ C_7H_8	59.99	4.96	6.18	4.19	59.60	4.96	6.02	3.76
14	$C_{16}H_{15}FN_2O_6$	54.86	4.32	8.00	5.42	54.75	4.16	7.78	5.49
22	$C_9H_{11}FN_6O_3$	40.00	4.10	31.10	7.03	40.07	4.16	31.10	6.99

Anti-HBV Activity

The ability of β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine compounds to inhibit the replication of HBV in a host can be evaluated according to any known method, including that below.

The antiviral evaluations were performed on two separate passages of cells, two cultures per passage (4 cultures total). All wells, in all plates, were seeded at the same density and at the same time.

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.0-fold (for HBV virion DNA) or 2.5-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are generally considered to be statistically significant [P<0.05] (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per cell basis in these experiments) were used

to calculate the levels of intracellular HBV DNA forms, thereby eliminating technical variations inherent in the blot hybridization assays.

Typical values for extracellular HBV virion DNA in untreated cells range from 50 to 150 pg/ml culture medium (average of approximately 76 pg/ml). Intracellular HBV DNA replication intermediates in untreated cells range from 50 to 100 pg/ug cell DNA (average approximately 74 pg/ug cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced, and occur more slowly, than depressions in the levels of HBV virion DNA.

For reference, the manner in which the hybridization analyses were performed for these experiments results in an equivalence of approximately 1.0 pg intracellular HBV DNA/ug cellular DNA to 2-3 genomic copies per cell and 1.0 pg of extracellular HBV DNA/ml culture medium to 3×10^5 viral particles/ml.

Toxicity analyses were performed in order to assess whether any observed antiviral effects were due to a general effect on cell viability. This can be assessed by the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV (herpes simplex virus) and HIV.

The test compounds were used in the form of stock solutions in DMSO (frozen on dry ice). Daily aliquots of the test samples were made and frozen at -20°C so that each individual aliquot would be subjected to a single freeze-thaw cycle. The daily test aliquots were thawed, suspended into culture medium at room temperature and immediately added to the cell cultures. The results are provided in Table 1.

Table 1: Anti-HBV Activity and Cytotoxicity of β-L-2'- and 3'-azido-5-FddC Compared to Lamivudine and L-5-FddC

Compound	Transfected 2.2.15 cells EC_{50} (μM) R.I.	Normal Hep G_2 cells CC_{50} (μM)
L-2-azido-5-FddC	0.1	>200
L-3-azido-5-FddC	0.01	>200
L-5-FddC	0.05	>200
Lamivudine (3TC)	0.03	>200

Example 3 Toxicity Of Compounds

The ability of the active compounds to inhibit the growth of virus in 2.2.15 cell cultures (HepG2 cells transformed with hepatitis virion) was evaluated. As illustrated in Table 1, no significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for any of the test compounds at the concentrations 100 mM.

Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as used for the antiviral evaluations. Each compound was tested at 4 concentrations, each in triplicate cultures. Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nM (A_{510}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{510} values (± standard deviations) in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. The percentage of dye uptake in the 9 control cultures on plate 40 was 100 ± 3. At 150-190 μM ß-D-ddC, a 2-fold reduction in dye uptake (versus the levels observed in untreated cultures) is typically observed in these assays (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

Example 4 Effect of anti-HBV β-L-deoxycytidine analogues on cell growth as assessed by human bone marrow clonogenic assays

The effects of anti-HBV β-L-deoxycytidine analogues on cell growth as assessed by human bone marrow clonogenic assays are shown in Table 2.

Table 2

Compound	CFU-GM EC_{50}	BFU-E EC_{50} (μM)
L-2'-azido-5-FddC	>10	>10
L-3'-azido-5-FddC	10	10
L-5-FddC	1.2	1.8
Lamivudine (3TC)	>10	>10
D-ddC (control)	0.7	0.05
Zidovudine (AZT) (control)	1.9	0.6

Preparation of Pharmaceutical Compositions

The compounds disclosed herein and their pharmaceutically acceptable salts, prodrugs, and derivatives, are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

Humans suffering from any of these conditions can be treated by administering to the patient an effective HBV-treatment amount of one or a mixture of the active compounds described herein or a pharmaceutically acceptable derivative or salt thereof, optionally in a

pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount without causing serious toxic effects in the patient treated.

A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art. In one embodiment, the active compound is administered as described in the product insert or Physician's Desk Reference for 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), or 2',3'-dideoxy-2',3'-didehydrothymidine (D4T) for HIV indication.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 mM, preferably about 1.0 to 10 mM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The active compound can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of the nucleosides that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example,

hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with cations such as sodium, potassium, zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylene-diamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N^6 or N^4 and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose,

gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, suspension, syrup, water, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV, anti-cytomegalovirus, or anti-HIV agents.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS). In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate,

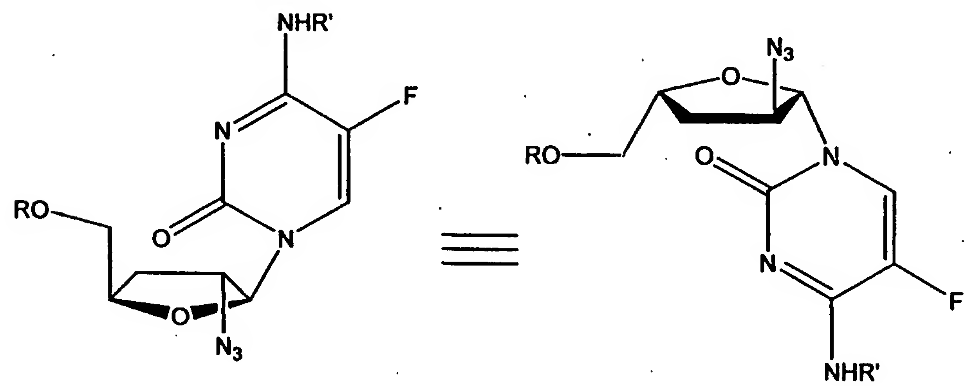
polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim:

1. A method for the treatment of hepatitis B virus infection in a host comprising administering an effective amount of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), R' is H, acyl, or alkyl.

2. The method of claim 1, wherein R is H.

3. The method of claim 1, wherein R is acyl.

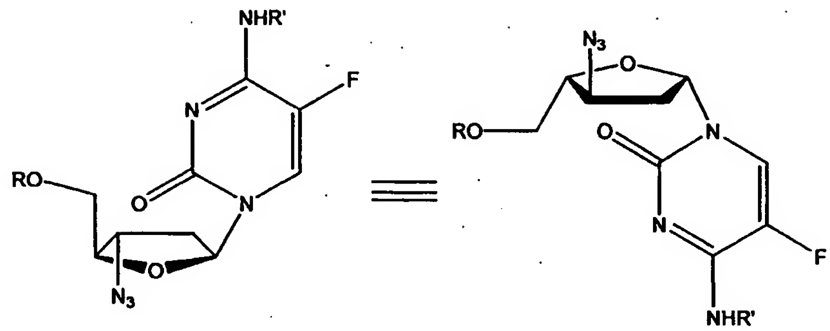
4. The method of claim 1, wherein R is monophosphate.

5. The method of claim 1, wherein R is diphosphate.

6. The method of claim 1, wherein R is triphosphate.

7. The method of claim 1, wherein R is a stabilized phosphate derivative.

8. A method for the treatment of hepatitis B virus infection in a host comprising administering an effective amount of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

9. The method of claim 8, wherein R is H.

10. The method of claim 8, wherein R is acyl.

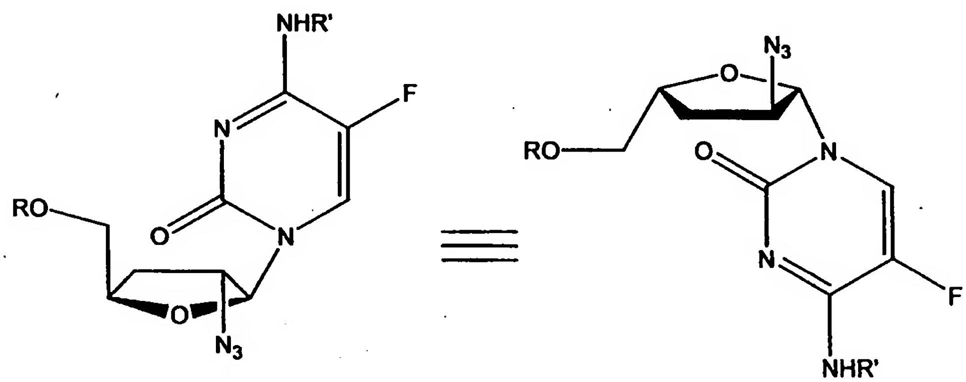
11. The method of claim 8, wherein R is monophosphate.

12. The method of claim 8, wherein R is diphosphate.

13. The method of claim 8, wherein R is triphosphate.

14. The method of claim 8, wherein R is a stabilized phosphate derivative.

15. A β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl

16. The compound of claim 15, wherein R is H.

17. The compound of claim 15, wherein R is acyl.

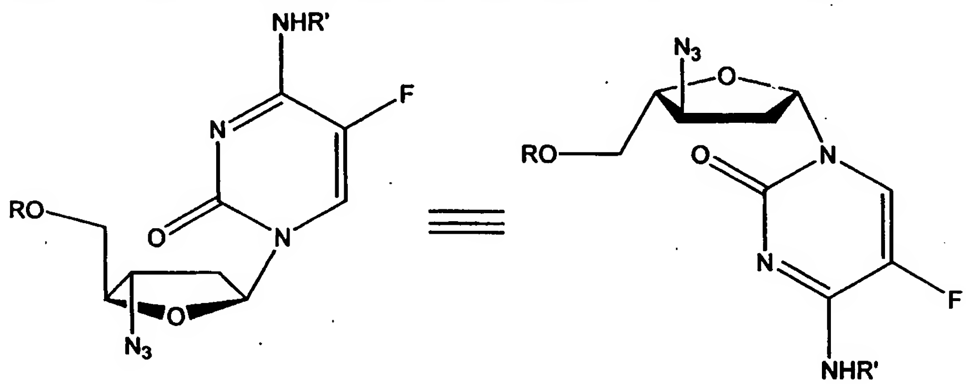
18. The compound of claim 15, wherein R is monophosphate.

19. The compound of claim 15, wherein R is diphosphate.

20. The compound of claim 15, wherein R is triphosphate.

21. The compound of claim 15, wherein R is a stabilized phosphate derivative.

22. A β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

23. The compound of claim 22, wherein R is H.

24. The compound of claim 22, wherein R is acyl.

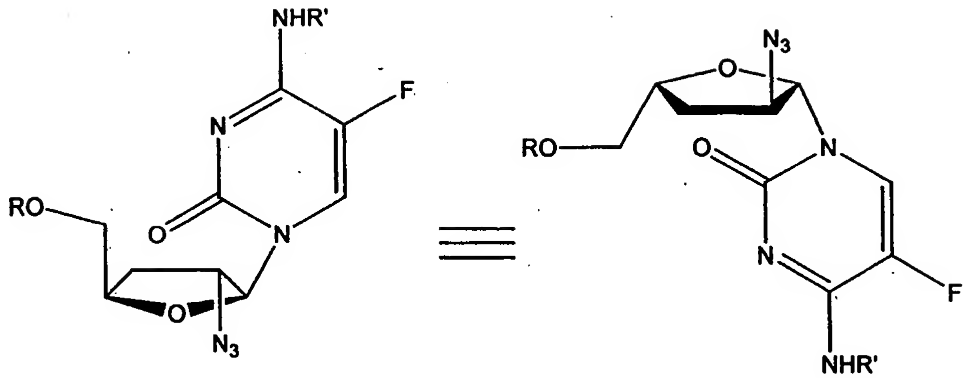
25. The compound of claim 22, wherein R is monophosphate.

26. The compound of claim 22, wherein R is diphosphate.

27. The comound of claim 22, wherein R is triphosphate.

28. The compound of claim 22, wherein R is a stabilized phosphate derivative.

29. A pharmaceutical composition for the treatment of hepatitis B virus infection in a host comprising an effective amount of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, in combination with a pharmaceutically acceptable carrier.

30. The composition of claim 29, wherein R is H.

31. The composition of claim 29, wherein R is acyl.

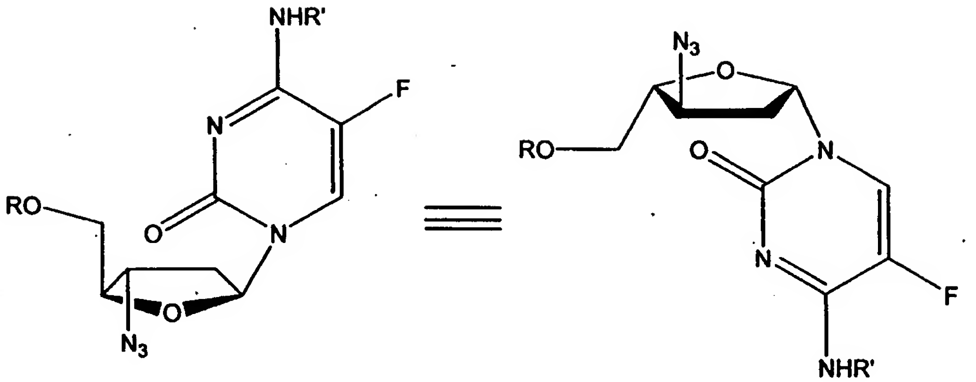
32. The composition of claim 29, wherein R is monophosphate.

33. The composition of claim 29, wherein R is diphosphate.

34. The composition of claim 29, wherein R is triphosphate.

35. The composition of claim 29, wherein R is a stabilized phosphate derivative.

36. A pharmaceutical composition for the treatment of hepatitis B virus infection in a host comprising an effective amount of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, in combination with a pharmaceutically acceptable carrier.

37. The composition of claim 36, wherein R is H.

38. The composition of claim 36, wherein R is acyl.

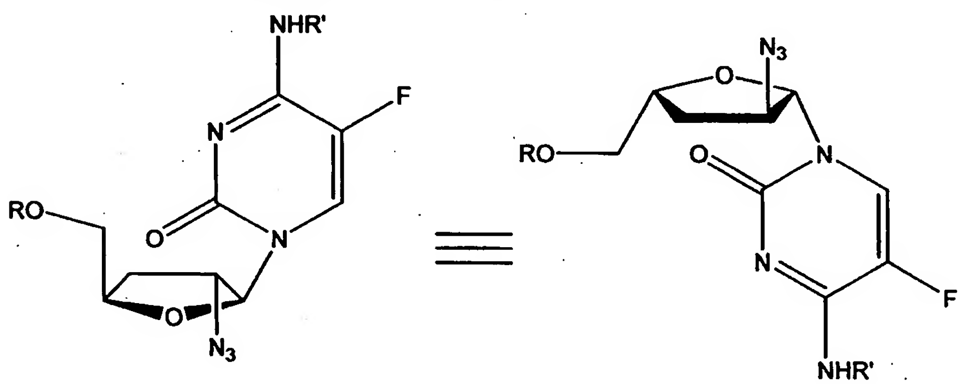
39. The composition of claim 36, wherein R is monophosphate.

40. The composition of claim 36, wherein R is diphosphate.

41. The composition of claim 36, wherein R is triphosphate.

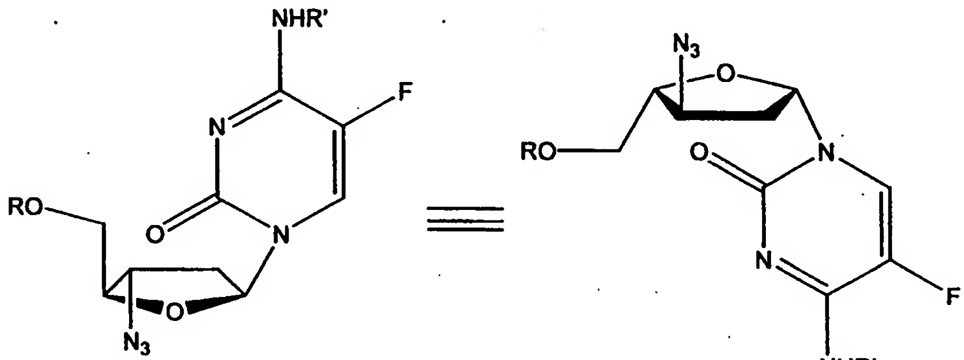
42. The composition of claim 36, wherein R is a stabilized phosphate derivative.

43. Use of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



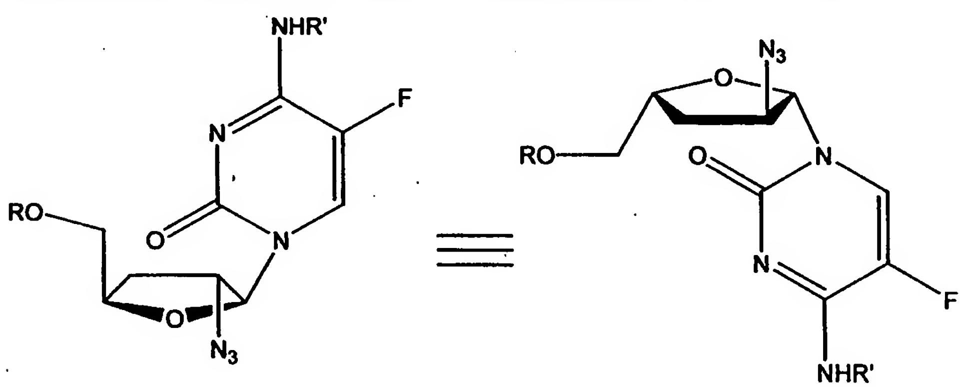
wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, for the treatment of hepatitis B virus infection in a human or other host animal.

44. Use of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



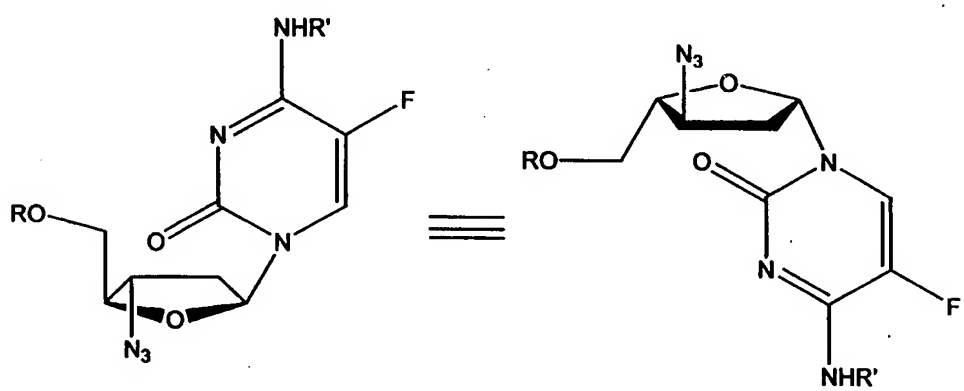
wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl for the treatment of hepatitis B virus in a human or other host animal.

45. Use of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, for the manufacture of a medicament for the treatment of hepatitis B virus infection in a human or other host animal.

46. Use of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl for the for the manufacture of a medicament for the treatment of hepatitis B virus in a human or other host animal.

47. The method of claims 1 or 8, wherein R is H and R' is H.

48. The method of claims 1 or 8, wherein R is acyl and R' is H.

49. The method of claims 1 or 8, wherein R is monophosphate and R' is H.

50. The method of claims 1 or 8, wherein R is diphosphate and R' is H.

51. The method of claims 1 or 8, wherein R is triphosphate and R' is H.

52. The method of claims 1 or 8, wherein R is a stabilized phosphate derivative and R' is H.

53. The method of claims 1 or 8, wherein R is H and R' is alkyl.

54. The method of claims 1 or 8, wherein R is acyl and R' is alkyl.

55. The method of claims 1 or 8, wherein R is monophosphate and R' is alkyl.

56. The method of claims 1 or 8, wherein R is diphosphate and R' is alkyl.

57. The method of claims 1 or 8, wherein R is triphosphate and R' is alkyl.

58. The method of claim 1 or 8, wherein R is a stabilized phosphate derivative and R' is alkyl.

59. The method of claim 1 or 8, wherein R is H and R' is acyl.

60. The method of claim 1 or 8, wherein R is acyl and R' is acyl.

61. The method of claim 1 or 8, wherein R is monophosphate and R' is acyl.

62. The method of claim 1 or 8, wherein R is diphosphate and R' is acyl.

63. The method of claim 1 or 8, wherein R is triphosphate and R' is acyl.

64. The method of claim 1 or 8, wherein R is a stabilized phosphate derivative and R' is acyl.

65. The compound of claims 15 or 22, wherein R is H and R' is H.

66. The compound of claims 15 or 22, wherein R is acyl and R' is H.

67. The compound of claims 15 or 22, wherein R is monophosphate and R' is H.

68. The compound of claims 15 or 22, wherein R is diphosphate and R' is H.

69. The compound of claims 15 or 22, wherein R is triphosphate and R' is H.

70. The compound of claims 15 or 22, wherein R is a stabilized phosphate derivative and R' is H.

71. The compound of claims 15 or 22, wherein R is H and R' is alkyl.

72. The compound of claims 15 or 22, wherein R is acyl and R' is alkyl.

74. The compound of claims 15 or 22, wherein R is monophosphate and R' is alkyl.

75. The compound of claims 15 or 22, wherein R is diphosphate and R' is alkyl.

76. The compound of claims 15 or 22, wherein R is triphosphate and R' is alkyl.

77. The compound of claims 15 or 22, wherein R is a stabilized phosphate derivative and R' is alkyl.

78. The compound of claims 15 or 22, wherein R is H and R' is acyl.

79. The compound of claims 15 or 22, wherein R is acyl and R' is acyl.

80. The compound of claims 15 or 22, wherein R is monophosphate and R' is acyl.

81. The compound of claims 15 or 22, wherein R is diphosphate and R' is acyl.

82. The compound of claims 15 or 22, wherein R is triphosphate and R' is acyl.

83. The compound of claims 15 or 22, wherein R is a stabilized phosphate derivative and R' is acyl.

84. The composition of claims 29 or 36, wherein R is H and R' is H.

85. The composition of claims 29 or 36, wherein R is acyl and R' is H.

86. The composition of claims 29 or 36, wherein R is monophosphate and R' is H.

87. The composition of claims 29 or 36, wherein R is diphosphate and R' is H.

88. The composition of claims 29 or 36, wherein R is triphosphate and R' is H.

89. The composition of claims 29 or 36, wherein R is a stabilized phosphate derivative and R' is H.

90. The composition of claims 29 or 36, wherein R is H and R' is alkyl.

91. The composition of claims 29 or 36, wherein R is acyl and R' is alkyl.

92. The composition of claims 29 or 36, wherein R is monophosphate and R' is alkyl.

93. The composition of claims 29 or 36, wherein R is diphosphate and R' is alkyl.

94. The composition of claims 29 or 36, wherein R is triphosphate and R' is alkyl.

95. The composition of claims 29 or 36, wherein R is a stabilized phosphate derivative and R' is alkyl.

96. The composition of claims 29 or 36, wherein R is H and R' is acyl.

97. The composition of claims 29 or 36, wherein R is acyl and R' is acyl.

98. The composition of claims 29 or 36, wherein R is monophosphate and R' is acyl.

99. The composition of claims 29 or 36, wherein R is diphosphate and R' is acyl.

100. The composition of claims 29 or 36, wherein R is triphosphate and R' is acyl.

101. The composition of claims 29 or 36, wherein R is a stabilized phosphate derivative and R' is acyl.

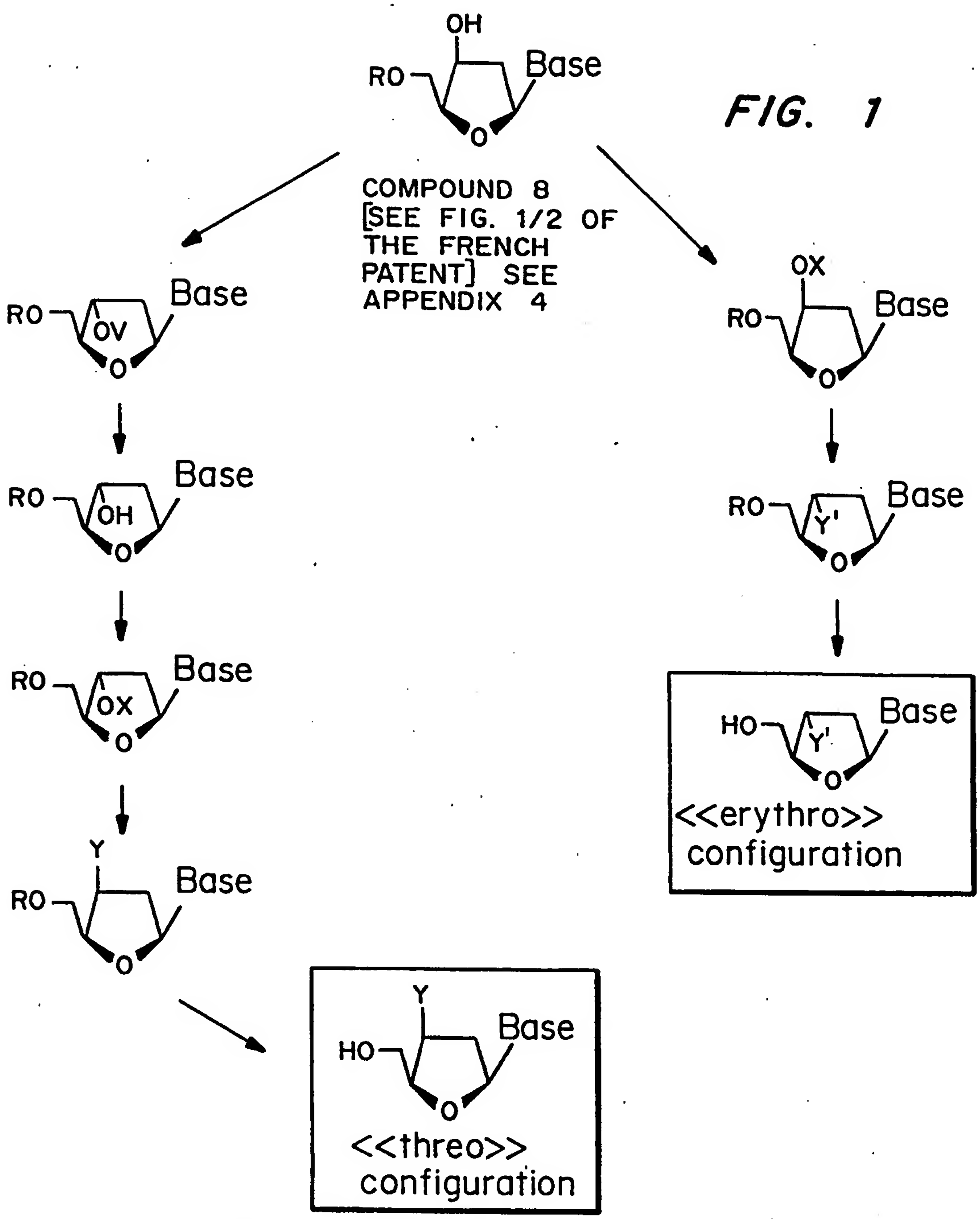


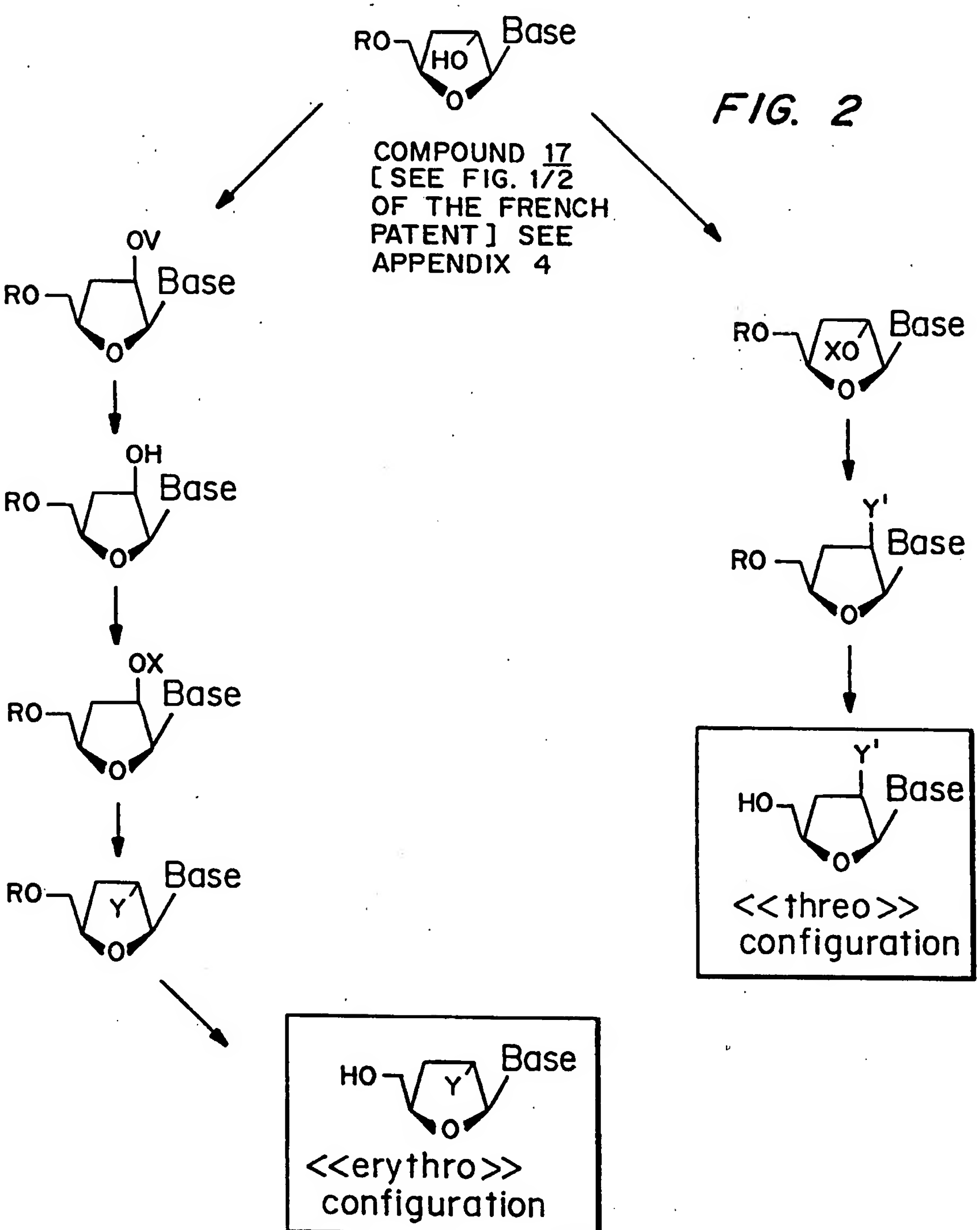
FIG. 1

V = acyl($CH_3-\overset{O}{\overset{\|}{C}}$, $C_6H_5-\overset{O}{\overset{\|}{C}}$)

X = Leaving group [CH_3SO_2, $CH_3C_6H_4SO_2$, CF_3SO_2]

Y,Y' = F, N_3, NR_1R_2 [R_1,R_2 = H, alkyl, aryl],
NO_2, NOR [R=H, alkyl, acyl], O-alkyl, O-aryl, etc.

FIG. 2



$V = \text{acyl}\ [CH_3-\overset{O}{\overset{\|}{C_6}}C_5H-\overset{O}{\overset{\|}{C}}]$

X = Leaving group [CH_3SO_2, $CH_3C_6H_4SO_2$, H, CF_3SO_2]

Y, Y' = F, N_3, NR_1R_2 [R_1, R_2 = H, alkyl, aryl],
NO_2, NOR [R=H, alkyl, acyl], O-alkyl, O-aryl, etc.

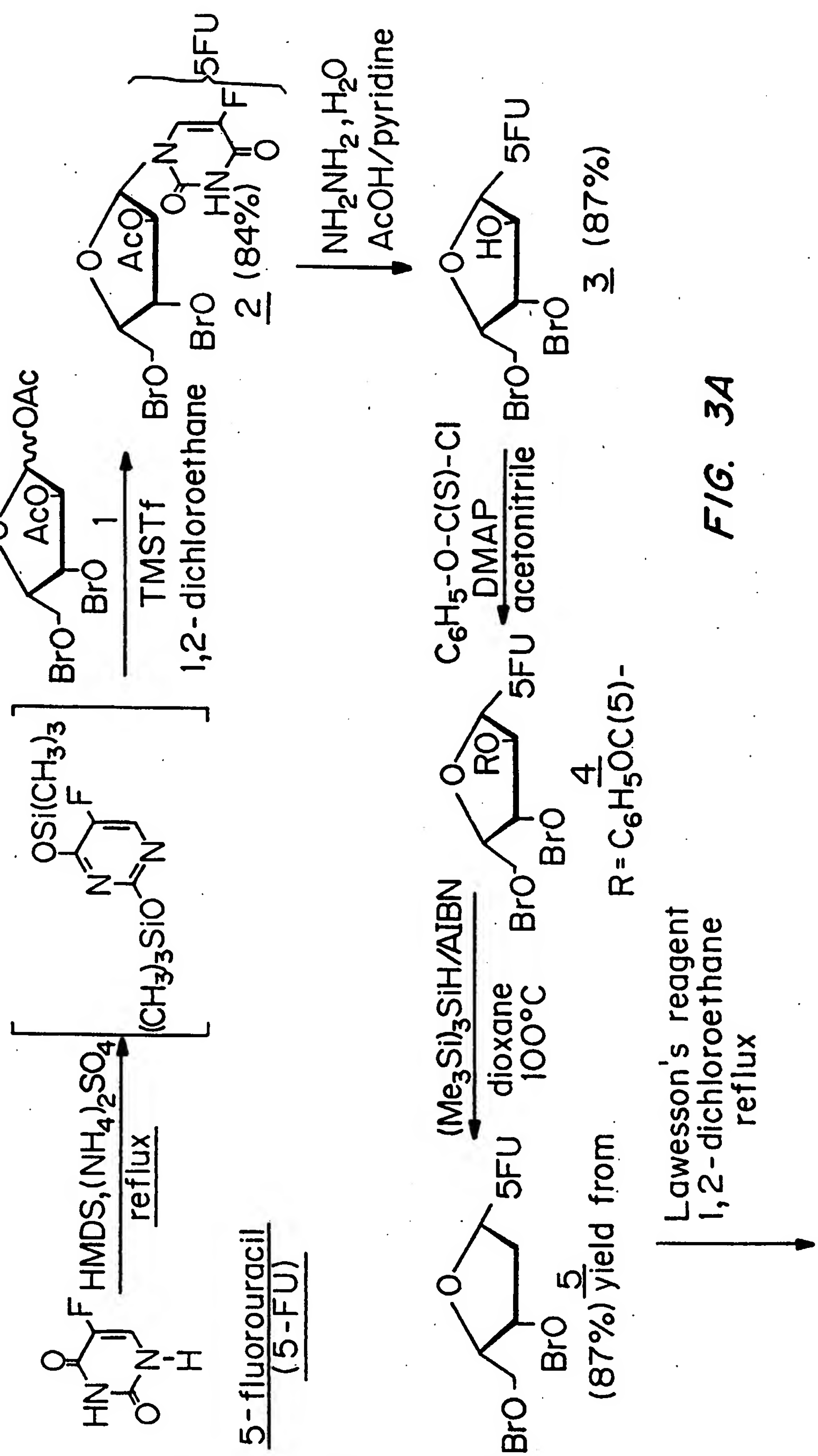


FIG. 3A

CONTINUED ON FIG. 3B

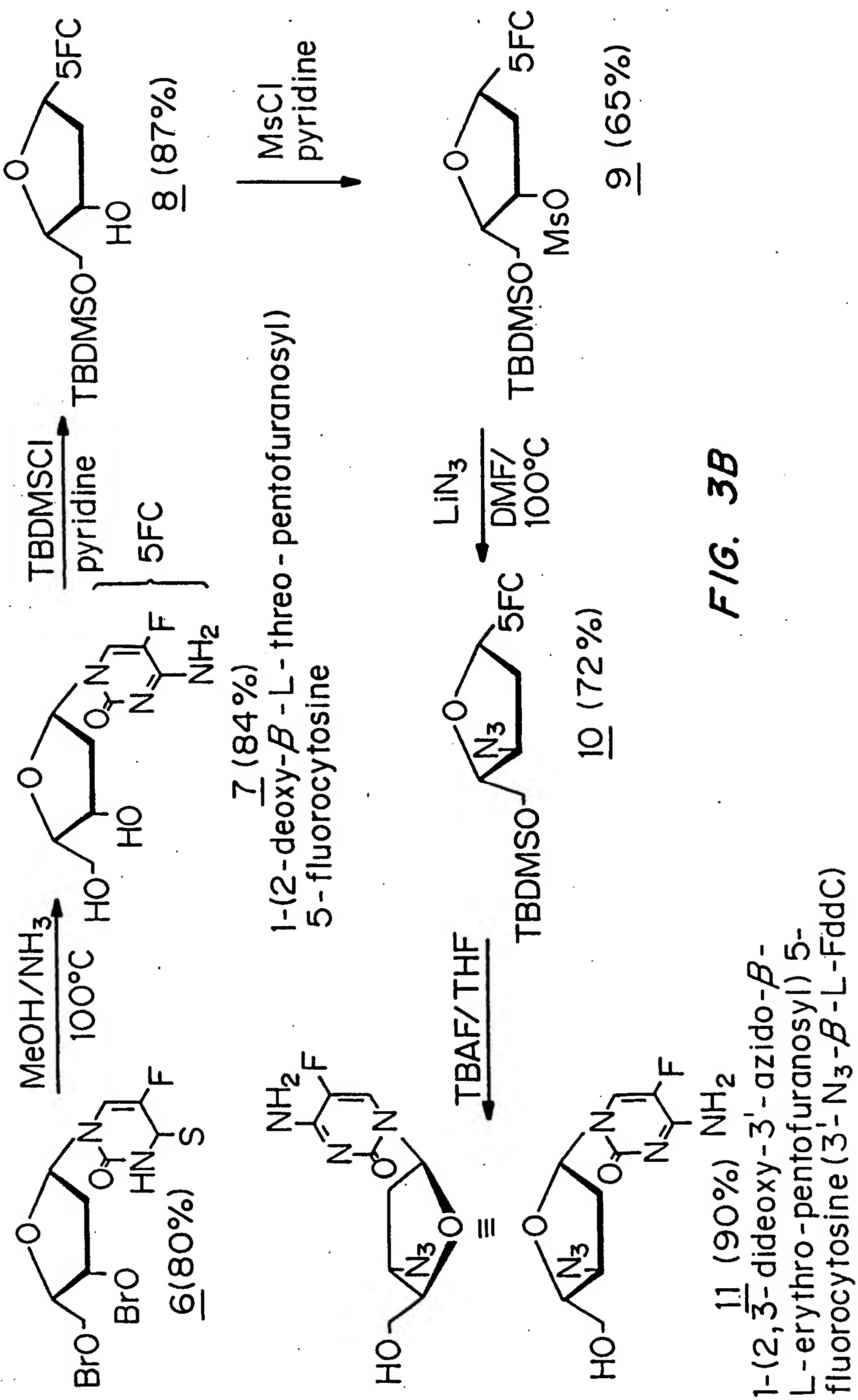

MeOH/NH3
100°C
TBDMSCl
pyridine
MsCl
pyridine
LiN3
DMF/
100°C
TBAF/THF
6(80%)
7 (84%)
1-(2-deoxy-β-L-threo-pentofuranosyl)
5-fluorocytosine
8 (87%)
9 (65%)
10 (72%)
11 (90%)
1-(2,3-dideoxy-3'-azido-β-
L-erythro-pentofuranosyl) 5-
fluorocytosine (3'-N3-β-L-FddC)

FIG. 3B

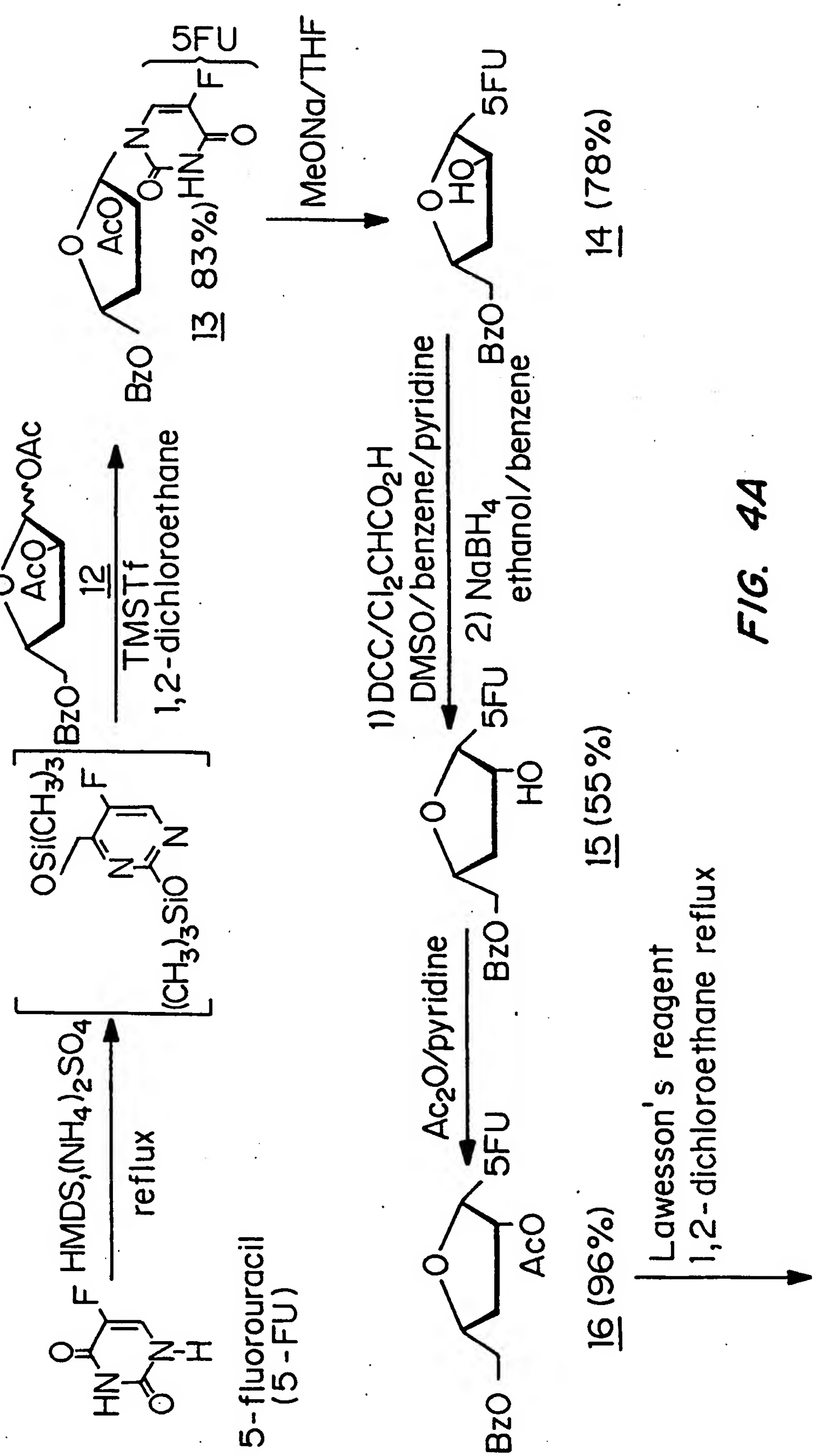


FIG. 4A

CONTINUED ON FIG. 4B

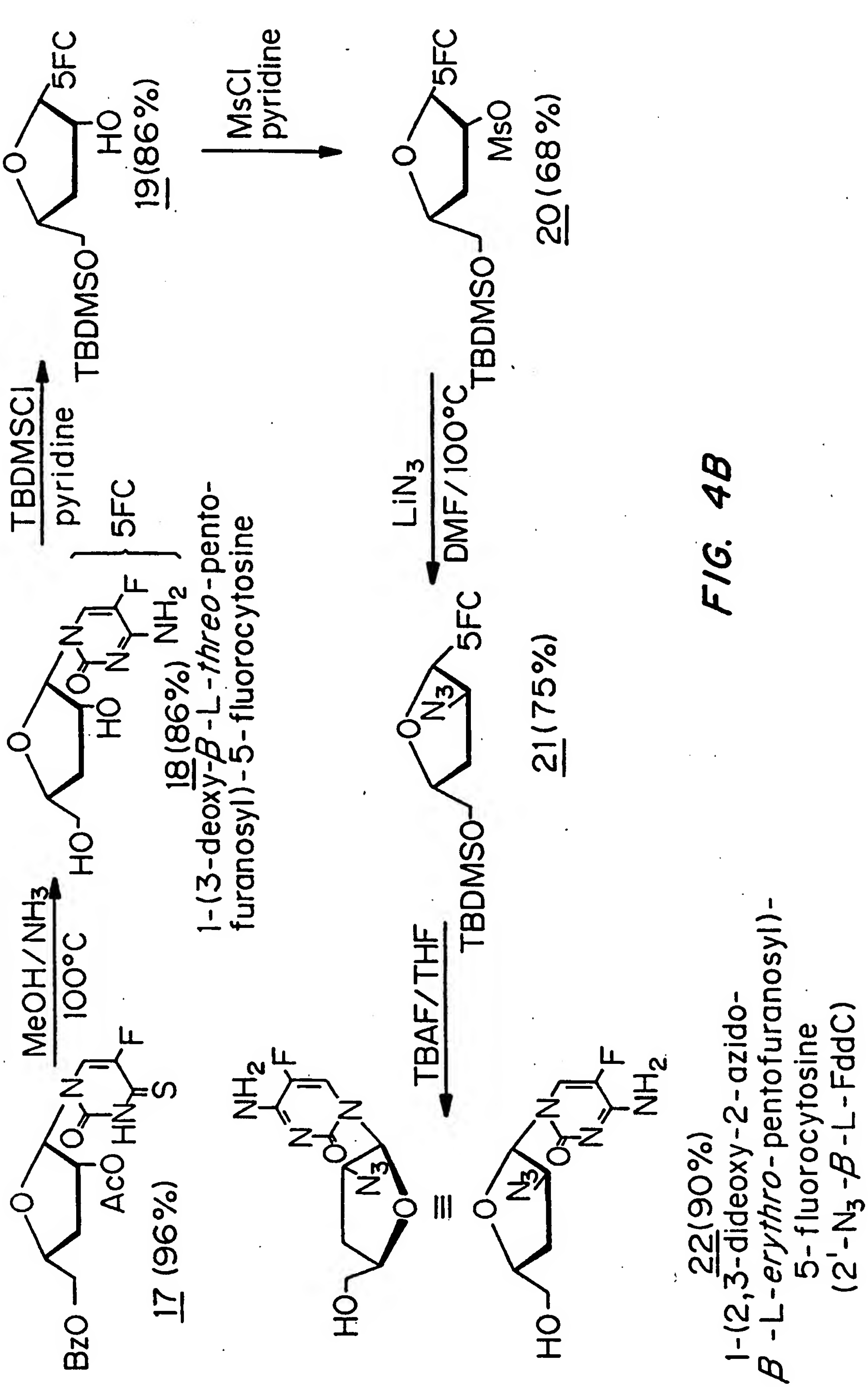

BzO
AcO
17 (96%)
MeOH/NH3
100°C
HO
18 (86%)
1-(3-deoxy-β-L-threo-pentofuranosyl)-5-fluorocytosine
5FC
TBDMSCl
pyridine
TBDMSO
19 (86%)
MsCl
pyridine
MsO
20 (68%)
LiN3
DMF/100°C
N3
21 (75%)
TBAF/THF
NH2
22 (90%)
1-(2,3-dideoxy-2-azido-β-L-erythro-pentofuranosyl)-5-fluorocytosine
(2'-N3-β-L-FddC)

FIG. 4B